ROTATION EFFECT OF PULSE CROPS ON NITROGEN FIXATION AND CARBON INPUT TO SOIL

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ABSTRACT

Pulse crops included in a crop rotation can reduce nitrogen (N) requirements via biological N_2 fixation (BNF) and provide greater carbon (C) inputs to soil than non-pulse crops in rotation. The goal of this research was to estimate the BNF and C input to soil by various pulse crops (chickpea, lentil and field pea) grown in rotation with pulse crops and non-pulse crops. Soil cores from three crop rotations (chickpea-wheat, lentil-wheat and pea-wheat) were collected from Swift Current, SK. Additional soil cores from two rotations (canola-wheat and wheat-canola) were extracted from a field used for commercial cropping in Central Butte, SK. The ^{15}N dilution method and continuous labelling with depleted $^{13}CO_2$ were used to estimate BNF and ^{13}C input to soil by pulse crops grown in a greenhouse. The continuous labelling with depleted $^{13}CO_2$ was effective in depleting ^{13}C in plants. The movement of ^{13}C from plant to soil C pools via rhizodeposition was also observed. However, an accurate amount of ^{13}C transferred was not measurable. Different pulse crop performed differently in rotation. Pea had the greatest amount of BNF and produced the most residue-C (pods, stems, leaves and roots) compared to chickpea and lentil. The crop grown in the first year of the three-year rotation also influenced the pulse crops grown in the third year of the rotation. Cropping the same first year and third year pulse crop in rotation (chickpea-wheat-chickpea and lentil-wheat-lentil) performed better than growing different first year and third year pulse crops in rotation (pea-wheat-chickpea and pea-wheat-lentil). Pulse crops grown immediately after wheat yielded better and fixed more N than those after canola. Growing a pulse crop after canola is not recommended in this soil zone.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-food Canada
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
C	Carbon
CNL	Canola
CP	Chickpea
HF	Heavy fraction
HSD	Honestly significant difference
N	Nitrogen
Na	Sodium
NaI	Sodium iodide
NH_4	Ammonium
NO_3	Nitrate
L	Lentil
LDPE	Low-density polyethylene
LF	Light fraction
P	Pea
PDB	PeeDee Belemnite
PVC	Polyvinyl chloride
SE	Standard error
SOC	Soil organic carbon
SOM	Soil organic matter

SPARC	Semiarid Prairie Agriculture Research Center
VLF	Very light fraction
W	Wheat
%Ndfa	Percentage of nitrogen derived from atmosphere

1. GENERAL INTRODUCTION

1.1 Introduction

Including a pulse in crop rotations has provided economic and environmental benefits to agricultural production in Saskatchewan. Policies and strategies that promote pulse crop usage have been encouraged because of their effect on mitigating greenhouse gas emissions and enhancing soil fertility. Growing pulses crops provides benefits by reducing N fertilizer input and helping to sequester C in soil (Lemke et al., 2007; Gan et al., 2011a).

Nitrogen is frequently the most limiting nutrient, and the nutrient applied in the largest quantity in agricultural production. Due to increasing demands for N fertilizer, the price of N fertilizer has increased dramatically over the past decades. Consequently, pulse crops have become a popular option in crop rotations due to their ability to reduce N fertilizer requirements. Pulse crops can acquire a high proportion of their total N needs from BNF (Walley et al., 2007). In addition to the reduced reliance on N fertilizer during growth, pulse crops also provide N benefits to the succeeding crops due to N sparing in the year that the pulse crop is grown followed by decomposition and release of N from pulse crop residues. The inputs of N to soil from pulse crops is comprised of N released via root rhizodeposition during crop growth and decomposition of root and aboveground residues. The quantity and quality of residue regulates the pattern and rate of N mineralization from crop residues (Lupwayi and Kennedy 2007), and varies among crop species. Arcand et al. (2013a) found that belowground N (N rhizodeposits and root N) accounts for 61% and 70% of total crop residue for pea and canola. In addition, the proportion of root-derived N in the soil inorganic N pool under pea (13%) was greater than under canola (4%). In order to capitalize on the N benefits of pulse crops, selection and frequency of cropping pulse crop in rotation has become increasingly important. In the study reported by Knight (2012), pea included every second year in rotation (alternating wheat-pea) fixed less N_2 than rotations where pea was included every three or four years. In two out of the three years of

the study, the highest BNF occurred in the most diverse rotation where pea was grown with the lowest frequency (canola-wheat-pea-wheat). In the other year of the study, the highest amount of N_2 fixed by pea occurred in the second most diverse rotation (pea-canola-wheat). It is unknown if this difference in BNF is solely attributed to the increased diversity of the rotation or to including canola in the rotations. It is also not known if pulse crops other than pea (i.e., chickpea and lentil) are similarly impacted by rotation sequence.

Recent research has shown that pea and lentil input more C into soil relative to canola and wheat (Glenn et al., 2011), thus conferring C sequestration benefits. Furthermore, including pulse crops in rotation may result in a more efficient conversion of residue C to SOC compared with monoculture wheat (Drinkwater et al., 1998; Campbell et al., 2000). Compared to cereal and canola residue, pulse residue C more effective in helping mitigate soil organic C (SOC) loss (Lemke et al., 2007). Pulse crops interact with SOC pools through rhizodeposition occurring during crop growth and the input of residue following crop harvest. In particular, SOC is strongly associated with the quality of crop residues returned to the field (Campbell et al., 2000). Comeau (2012) found that even though root production of pulse crops was less than shoot production, the root residues and shoot residues contributed equally to the SOC status. In addition to root residue, rhizodeposits from the roots also account for a considerable proportion of belowground residue that may influence SOC status (Gan et al., 2010; Comeau, 2012). Therefore, it was the goal of my project to evaluate C input derived from pulse crop residues specifically from roots and rhizodeposits to SOC as influenced by cropping sequence.

A novel method of tracing C in plant-soil system was used in this study. Plants were labelled with depleted $^{13}CO_2$, a source of CO_2 produced from propane. Compared to traditional enriched $^{13}CO_2$, depleted $^{13}CO_2$ is less expensive and the labelling itself is less labour intensive. The effectiveness of this method is examined in this study.

By comparing BNF and C inputs under three pulse crops (chickpea, lentil and pea), and any effect of rotation sequence, the most efficient crop combination to maximize N fixation and C storage may be estimated. The most efficient utilization of BNF will reduce the N fertilizer requirement for subsequent crops. As well, the most efficient crop combination will confer SOC benefits to crops grown after the pulse.

The overall objectives of this study were to: 1) determine BNF on chickpea, lentil and pea as influenced by cropping sequence; 2) evaluate the effectiveness of a new method which uses continuous labelling with depleted $^{13}CO_2$ to trace ^{13}C in the plant-soil system; 3) estimate C input of pulse crop (chickpea, lentil and pea) residues to soil C pools as influenced by cropping sequence.

1.2 Organization of the thesis

The research presented in this thesis was organized in manuscript format. Following this introduction (Chapter 1) was the literature review (Chapter 2). Two studies were presented in Chapter 3 and Chapter 4. The goal of Chapter 3 is to examine the effect of cropping sequence on BNF of chickpea, lentil and pea using dilution ^{15}N isotope method under controlled conditions in a greenhouse. The objective of Chapter 4 is to examine the cropping sequence effect on C input to soil from three pulse crops (chickpea, lentil and pea) to soil using continuous labelling with depleted $^{13}CO_2$. This chapter also evaluates the effectiveness of this continuous labelling with depleted $^{13}CO_2$. Chapter 5 is the synthesizes the major findings of the research studies and suggests future work. Finally, Chapter 6 is comprised of a list of the literature cited.

2. LITERATURE REVIEW

2.1 Prairie cropping systems

Cereal-fallow rotations were dominant in western Canadian from the turn of the century until the 1960s. These monoculture cropping systems were dependent on extensive use of mechanical tillage for weed control and seed-bed preparation (Zentner et al., 2002). The extensive use of tillage contributed to soil degradation and lowered the soil's resistance to further erosion losses and reduced conserved soil moisture (Arcand et al., 2013b; Zentner et al., 2002). Fallow was used to retain soil moisture and accumulating soil available nutrients for subsequent crops (Comeau, 2012). From the late 1980s, the use of extended and diversified crop rotations, together with adoption of no-tillage or minimum-tillage practices, have gradually replaced the traditional cereal monoculture. By retaining crop residues on the soil surface, no-tillage management improves soil moisture retention, allowing producers to grow crops in the time and space previously used for summer fallow (Grant et al., 2002). In addition, the introduction of diverse crops helps to control weeds, pests and diseases, and improves soil nutrient management (Omnski et al., 1999; Zentner et al., 2001). Although wheat remains the dominant field crop on the Prairies, the inclusion of oilseed and pulse crops in crop rotations with cereals has gained widespread acceptance among producers.

The cool climatic conditions of the Canadian prairies are suitable for the growth of oilseed crops, including the *Brassica* species, canola, mustard and flax (Gan et al., 2004; Johnston et al., 2002). Canola is the dominant oilseed crop in Canada and the second most widely sown crop in Saskatchewan (Johnston et al., 2002; Statistics Canada, 2009). Canola was developed by plant breeders in Saskatchewan during the 1960s, and the crop has increased from 2 million to 8 million hectares grown throughout the country during the past 30 years (May et al., 2010, Statistics Canada, 2009). Canola was often grown in rotation with wheat, oat and barley (Statistic Canada, 2009). The effect of a preceding crop of canola on subsequent crop yield may be

positive or negative (Grant et al., 2009; Koide and Peoples, 2012). For examples, decreased yields may be related to soil growing conditions. Canola may reduce soil organic matter and populations of beneficial microorganism populations such as rhizobia in following crops when the land is frequently cropped to canola (Vera et al., 1987). Another soil-related problem with short-term canola rotations is that compared to cereals, canola crop residues usually decay more rapidly, which means diminishing amounts of residue lead to a less protected soil surface with a greater probability of soil erosion (Canola Council of Canada, 2014).

Pulse crops are legume crops and their seed is produced for human consumption, and are a relatively recent addition to crop rotations on the Canadian prairies. The main species currently cultivated in Canada are: field pea, lentil, chickpea, faba bean and dry bean. Canada is the No.1 exporter of pulse crops in the world (Arcand, 2013a). Although pulse crops occupy a less dominant role than wheat and canola, they are of particular research interest in crop rotations due to their ability to fix atmospheric N.

Pulse crops are considered to be a great contributor to and diversifier of various crop rotations. The major economic and environmental benefits associated with pulse crops in rotation are: reducing use of non-renewable energy (Hardarson and Atkins, 2003; Zentner et al., 2004; Saskatchewan Research Council. 2011), decreasing carbon footprints (Lemke et al., 2007; Dusenbury et al., 2008; Gan et al., 2011a; Gan et al., 2011b), improving yield potential (Miller et al., 2002; Gan et al., 2003; Miller et al., 2003; Miller et al., 2006) and enhancing soil fertility (Gan et al., 2002; Johnston et al., 2007; Lupwayi and Kennedy, 2007). These benefits are attributed to various factors. For example, because pulse crops can fix N through BNF, including a pulse crop in rotations can reduce dependence on N fertilizer. In addition, including a pulse crop in rotation can decrease disease (Krupinsky et al., 2002) and diminish weed populations for the following crops (Seymour et al., 2012). Increased SOC, enhanced soil nutrients and improved soil structure may occur due to the return of crop residues (Grant et al., 2002; Lupwayi and Kennedy, 2007). Therefore, the selection of crops and their sequence need to be considered in diversified rotations.

2.2 Pulse crops

2.2.1 Biological N_2 fixation

Nitrogen is essential for plants. It is a key element for forming protein, amino acids and genetic material. Furthermore, though N is a relatively common element, it is also one of the most limiting nutrients for crop production. Nitrogen in the atmosphere comprises 78% of the air. Approximately 90% of the atmospheric N entering the biosphere is a result of BNF. Biological N_2 fixation, discovered by Beijerinck in 1901 (Beijerinck, 1901), is carried out by specialized groups of organisms that convert atmospheric N_2 to ammonium (NH_4) catalyzed by the nitrogenase enzyme. In leguminous plants, rhizobia invade the plant roots and form nodules where BNF takes place (Havlin et al., 2005). The rhizobia supply fixed N_2 to the plant in exchange for the plant providing carbohydrates to the rhizobia.

The amount of N_2 fixed by crops varies, for example, pulse crops can be ranked according to their estimated ability to fix N_2: faba bean>pea>chickpea>lentil >dry bean (Walley et al., 2007). Similarly, Gan et al. (2010) and Herridge et al. (2008) reported that global averages for the amount of N fixed by pulse crops commonly grown on the Canadian prairies are 23, 51, 58, 86 and 107 kg N ha^{-1} for common bean, lentil, chickpea, field pea and faba bean, respectively. The percentage of N derived from atmosphere (%Ndfa) is crucial for determining the amount of N fixed (Gan et al., 2010; Soon and Lupwayi, 2008). The %Ndfa also varies in crops. Walley et al. (2007) reported the median percentage of %Ndfa in field pea (55%), lentil (60%) and chickpea (55%). The % %Ndfa can be as low as 0%, to as high as 81% partly depending on the effectiveness of the symbioses (Soon and Lupwayi, 2008).

Establishing a symbiotic N_2-fixing association includes two main stages: root hair infection and nodule organogenesis. During the root hair infection process, the plant root sends signals by exuding various substances. Rhizobia sense the signal and travel relatively close to contact with the root hair. Once bound to the root hair, rhizobia respond by producing their own signals called Nod factors, which deform the root hair to trap rhizobia (Walley, 2013). Rhizobia then invade the root hair through formation of an infection thread. The infection thread is “an intercellular tube that penetrates the cells of the plant, and the bacteria then enter the root cells through the deformed root hair” (Wang et al., 2012). The infection thread continues to develop from cell to cell, extending into the inner cortex of the root. The bacteria multiply within the expanding

network of tubes and continue to produce Nod factors which stimulate the root cells to proliferate, eventually forming a root nodule. The bacteria continue to divide, and induce the establishment of the N_2-fixing enzyme system including the synthesis of nitrogenase in bacteroids, which are root nodules colonized by thousands of living rhizobia (Downie, 2010). Biological N_2 fixation occurs in symbiosomes, which may contain several or just one bacteroid (Udvardi and Day, 1997). Thus, the symbiotic relationship is established between rhizobia and a host legume plant. Rhizobia can provide N resources to the plant and in exchange, the plant provides carbohydrates to rhizobia.

The effectiveness of legume and rhizobia symbiosis is a major contributor to BNF (Mohammadi et al., 2012). Establishment of effective symbioses is a complex process, which can be impacted by specificity factors, such as host rhizobia strains and cultivars (Al-Falih, 2002; Tripath and Psychas, 1992; Vessey, 2004; Soon and Lupwayi, 2008; Abi-Ghanem et al., 2011; Mut et al., 2012). Specificity genes determine which *Rhizobium* strain can infect which legume (Al-Falih, 2002). Even though a strain can infect a legume, the nodules may not be able to fix N_2. Only effective strains can induce N_2 fixing nodules (Walley, 2013). More effective strains can improve rhizobia infection and efficiency, thereby increasing BNF (Vessey, 2002; Abi-Ghanem et al., 2011). In addition to rhizobia strains, host crop varieties can strongly impact BNF (Mut et al., 2012). Hafeez et al. (2000) reported 42% variability in BNF among various rhizobia strains, and 81% variability among different varieties of lentil. Cultivar and strain interactions are strongly associated with BNF among legume species (Israel 1981; Valverde and Otabbong 1997; Kellman, 2008). Crop variety and rhizobia strain variety interactions are also significant factors impacting BNF, and may have more effect than strain alone (Mud et al., 2012).

External environment factors and management also can impact BNF. Soil microbial populations (Slattery et al., 2001; Al-falih, 2002; Mohammadi et al., 2012; Walley, 2013), cropping sequence (Matus et al., 1997; Slattery et al., 2001; Kellman, 2008), soil N availability (Walley et al., 2007; Kellman, 2008; Mohammadi, 2012) and plant density significantly affect BNF of pulse crops (Ayaz et al., 2004; Strydhorst et al., 2008; Mohammadi; 2012).

Rhizobia need to compete with other soil organisms, including other bacteria, fungi and protozoa. Competition for limited supplies of nutrients is an important interaction among soil microbial communities. Some *Rhizobium* general grow slowly and may not be effective

competitors in soil (Al-falih, 2002). The main effect of soil organisms in the legume rhizosphere is alteration of the size and composition of the rhizobia population, which may lead to a numerical advantage for a certain rhizobial strain, eventually altering nodule occupancy (Al-falih, 2002). Rhizobia survival and infectivity can be influenced by crop rotation (Slattery et al., 2001). Decomposing crop residues can release biocidal compounds, which affect soil microorganisms (Augus et al., 1994). Muehlchen et al. (1990) reported that nodulation of field pea was reduced following a mustard crop, which may relate to reduced ability of the *Rhizobium* to survive in the soil.

Available inorganic N is one of the most important factors impacting BNF. High soil available N levels inhibit the *Rhizobium* infection process, thereby inhibiting the BNF of pulse crops due to diversion of photosynthesises towards assimilation of nitrates (Tripath and Psychas, 1992; Viosin et al., 2002; Boldsaikhan, 2010). Voisin et al. (2002) also reported that low inorganic N level before growing in soil can stimulate early root growth, contributing to enhanced nodulation and BNF.

Plant density can affect BNF. In the absence of weed competition, pulse seed and BNF yields were increased with a higher plant density (Strydhorst et al., 2008). The explanation was that increasing the competition for soil N through increased pulse plant density contributed to increases in the proportion of N in plant derived from BNF (Danso et al., 1987). Materon and Danso (1991) found plant density did not influence %Ndfa; however, the total amount of N_2 fixed was increased because of higher dry matter and total N yields.

2.2.2 Pulse crop residue

Pulse crop residues can confer N and non-N benefits to subsequent crops. Since pulse crops can obtain a high proportion of their total N requirements from BNF, N fertilizer requirements in pulse-cropping systems decrease. In these systems, the N benefit to the following crops depends on the decomposition and release of N from the pulse crop residues (Jensen, 1996). The crop residue inputs to soil comprise of aboveground residues (pods, stems and leaves) and belowground residues which includes roots and rhizodeposits released from roots.

Compared to cereal and oilseed crops, the availability of N for subsequent crops through mineralization is higher from pulse residues. Pulse crop residues remaining after harvest have a

different biochemical composition (reflected in the C:N ratios), compared to other crop residues (Lupwayi and Kennedy, 2007). In soil, microorganisms are responsible for N and C cycling (Bengtsson et al., 2003; Gan et al., 2011b). For microorganisms, C is a source of energy and is basic building block of life, and N is necessary for genetic material, proteins and cell structure. Generally, residues with a narrow C:N ratio (20:1) promote mineralization, whereas residues with a wide C:N (more than 40:1) immobilize N. Microorganisms need a C:N ratio high enough to provide energy while also supplying N for building compounds. If lots of energy (C) but not enough N (wide C:N ratio) then all of the N is captured in the microbial biomass and used by the organism directly (Comeau, 2012). If lots of N relative to C then C is used for energy, while N in excess of what is needed is N released into soil for N uptake (Gan et al., 2011a). Therefore, residues with wide C:N ratios are considered to be low quality resources due to their low mineralization potential. Pulse crop residues are considered high quality which they have a C:N ratio between 25:1 to 40:1 (Stevenson and van Kessel, 1996; Soon and Arshad, 2002; Brady and Weil, 2008). Canola and wheat are of medium quality (Soon and Arshad, 2002; Brady and Weil, 2008). The C:N ratio for cereal above-ground residues typically range from 70:1 to 100:1 (Stevenson and van Kessel, 1996). The C:N ratio of canola above-ground residues ranges widely from 71:1 to 132:1 (Soon and Arshad, 2002; Bhupinderpal-Singh et al., 2006).

The potential non-N benefits of crop residue include increased soil organic matter (SOM) and improved soil structure (Grant et al., 2002; Vanden Bygaart et al., 2003), because including pulse crops in rotations helps to maintain SOC and fertility (Lupwayi and Kennedy, 2007). The quantity and quality of crop residue returned to the land impacts the status of SOC (Campbell et al., 1997). Although pulse crops produce less biomass and return less total C to the soil, pulse crop residues can be converted more efficiently into SOC than cereal and canola (Comeau, 2012). Surprisingly, the low amount of pulse crop residue with low C:N ratio can produce more SOC than a high amount of crop residue having a high C:N ratio (Comeau, 2012).

2.3 Methodology to estimate BNF and follow C dynamics using isotope tracers

2.3.1 ^{15}N isotope technique

The main stable isotopes of N are ^{14}N and ^{15}N, and ^{14}N is more naturally abundant than ^{15}N. Two stable ^{15}N techniques are widely used to estimate BNF: ^{15}N natural abundance and ^{15}N isotope dilution. The principle of these techniques is that N_2 fixing plants have different amounts of ^{15}N in their tissues than non-fixing plants (Delwiche and Stein, 1970; Unkovich et al., 2008). Based on the difference in the $\delta^{15}N$ signatures, it is possible to estimate BNF on the basis of ^{15}N analyses from the N_2-fixing plant and a non-fixing reference plant.

The ^{15}N natural abundance technique relies on naturally-occurring differences in ^{15}N abundance between available soil N and that of atmospheric N_2 (Hauck 1973; Rennie et al., 1976; Warembourg, 1993; Unkovich et al., 2008). This is caused by the difference between the source of plant N in N_2 fixing and non-fixing crops. When an effectively nodulated plant uses a combination of N from atmospheric N_2 and soil mineral N, or when it only uses BNF for growth, the $\delta^{15}N$ values of the plant would resemble that of atmospheric N_2. Conversely, a non-fixing plant can take up only soil available N; therefore its ^{15}N signature should be similar to that of the soil mineral N. The assumption of this method is that the ^{15}N signature of non N_2-fixing plants is identical to the ^{15}N signature of soil utilised by the N_2-fixing crops.

Different from the natural abundance method, the isotope ^{15}N dilution method involves growing both N_2 fixing and non-fixing crops in soil fertilized with the same amount of ^{15}N labelled fertilizer (Legg and Sloger, 1975; Rennie, 1982; Hardarson and Danso, 1990; Unkovich et al., 2008). When actively fixing atmospheric N_2 in legume crops, the ^{15}N enrichment is diluted by atmospheric N_2. The principal assumption is that ^{15}N enrichment of the control non-fixing crop equals the ^{15}N enrichment of the N derived from the soil in the N_2 fixed crop (Unkovich et al., 2008). In this respect, the ^{15}N enrichment of the soil N needs to be relatively constant over time, or soil N uptake by the N_2-fixing and non-fixing crops need to be the same. However, the mineralization of unlabelled N can contribute to an unstable ^{15}N enrichment of soil mineral N over time (Viera-Vargas et al., 1995). In addition, soil mineral N uptake patterns vary among plant species, making it difficult to choose an ideal non-N_2 fixing reference plant (Unkovich and Pate, 2000; Peoples and Herridge, 1990). Although its ^{15}N dilution method is considered the most accurate method to estimate %Ndfa of legumes in a field, it remains subject to error.

Selection of a suitable non-fixing crop as the reference crop may determine the accuracy of the techniques (Boldsaikhan, 2010). The use of different reference crops can produce a range of different estimates of BNF (Haynes et al., 1993). For example, Haynes et al. (1993) reported that a higher %Ndfa when using barley (*Hordeum vulgare L.)* compared to perennial ryegrass (*Lolium perenne L.*) as the non-fixing reference crop. To increase the likehood of the reference and fixing crops have identical $\delta^{15}N$ values, the reference crop should have a similar pattern of soil N uptake as the fixing crop (Rennie, 1986; Witty, 1983). In addition, periods for planting and harvesting of fixing and reference crops should be similar (Rennie, 1983). Reference crops should exploit the same soil N pool as fixing crops (Chalk, 1985).

2.3.2 ^{13}C labelling method

Three isotopes of C occur naturally: ^{12}C accounts for 98.9% of the C, ^{13}C is 1.1% of total C, and ^{14}C only makes up about 1 part per trillion (1×10^{-9}%) of the C in the atmosphere. Natural variations in ^{13}C and ^{14}C abundance are usually expressed in terms of δ units, which are parts per thousand (‰) deviation relative to the standard of an established reference material. The established reference material is PeeDee Belemnite (PDB), which has a standard $^{13}C/^{12}C$ ratio (0.0112372) (Graig, 1957). By convention, PDB has a $\delta^{13}C$ value of 0‰. Materials with positive $\delta^{13}C$ values are enriched in ^{13}C, those with negative values depleted in ^{13}C relative to the PDB standard. Generally, $\delta^{13}C$ values range from -23 to -40‰ in C_3 plants (i.e. pulse and wheat), with a median value of about -27‰ (Balesdent and Mariotti, 1996). Atmospheric CO_2 has a $\delta^{13}C$ value of (-6 to -8‰) (Balesdent and Mariotti, 1996). ^{14}C is a radioactive isotope, therefore ^{14}C does not occur naturally in plants.

Plants that have been labelled with ^{13}C or ^{14}C can be used to trace plant residue decomposition through different SOM pools. Since the 1960s, ^{14}C has been widely used in laboratories to study aspects of SOM dynamics (Warembourg and Kummerow, 1991; Meharg, 1994). Because there is no ^{14}C in naturally grown plants, all ^{14}C in labelled plants originates from labelled atmospheric $^{14}CO_2$. A study by Stevenson (1986) showed that by labelling a plant with ^{14}C materials it was possible to examine newly added SOM from plant C. However, because ^{14}C is a radioactive material which requires extensive precautions for handling, and thus, is rarely used in experiments conducted over one or two months (Bromand et al., 2001). More recently, ^{13}C has been used in place of ^{14}C because of its safety for handling (Bromand et al., 2001) and

improvements in analytical techniques (Recous et al., 2000). Methods for labelling plants with atmospheric $^{13}CO_2$ can be broadly classified as: (1) pulse labelling; (2) repeat-pulse labelling and (3) continuous labelling (Hanson et al., 2000).

Pulse labelling is accomplished by exposing plants to a single pulse of highly enriched $^{13}CO_2$ (usually 99 atom %$^{13}CO_2$) for a short period at some point during plant growth. Pulse labelling is ideally used for determining the fate of CO_2 in small plants grown in closed laboratory chambers, where all added C can be accounted for (Warembourg and Paul, 1973; Mehag and Killham, 1988; Cheng et al., 1993).

Repeat pulse-labelling systems involves exposing plants repeatedly to $^{13}CO_2$ during the growing season (Bromand et al., 2001). In repeat-pulse labelling, plants are usually exposed to a relatively less enriched $^{13}CO_2$ (33 atom %$^{13}CO_2$) atmosphere than pulse labelling. It is postulated that the label can be uniformly distributed in the plant by adjusting the amount of label applied in proportion to the change in photosynthetic rate over the growing season (Bromand et al., 2001). Repeat pulse labelling at regular intervals has been successfully used to estimate cumulative belowground C input (Jensen, 1993), to approximate the rhizodeposition rate (Thompson, 1996; Sangter, 2010), and to investigate plant C incorporation into different soil C pools (Hooker and Stark, 2012).

Continuous labelling involves exposing plants to a constant rate of labelled CO_2 over the life span of the plants. The main advantages of continuous labelling over pulse labelling are: (1) it provides a more homogenous labelling of plant C pools compared to pulse labelling, and (2) the kinetics of C incorporation are under steady state assumptions, which simplifies calculations (Kouchi and Yoneyama, 1984; Martin et al., 1992; Hanson et al., 2000). Continuous labelling can be used to estimate the amount of C transfer by the plant into the soil and below-ground pools (International Atomic Energy Agency, 2001).

The continuous labelling methods has been successfully used enriched $^{13}CO_2$ to estimate the amount of C transferred by the plants into soil and below-ground pools (Keel et al., 2006; Grams et al., 2010). Studer et al. (2014) reported a significant enrichment in ^{13}C in SOM occurred compared to the natural isotope background after 14 days of continuous labelling with enriched $^{13}CO_2$ (with 10 atom% $^{13}CO_2$). However, the high cost of the ^{13}C-enriched CO_2 makes it

unsuitable for large experiments or for continuous labelling over a long period. A continuous labelling with depleted $^{13}CO_2$ method has been proposed for tracing C in plants through photosynthesis (Cheng et al., 2005). This method uses commercially available, inexpensive CO_2 produced from natural gas which has a $\delta^{13}C$ value ranging from -40‰ to -55‰ (Cheng et al., 2005). Thus, when plants fix this CO_2 through photosynthesis, the $\delta^{13}C$ value in the labelled plants is reduced rather than enriched. Compared to continuous ^{13}C enrichment, the continuous labelling with depleted $^{13}CO_2$ requires less equipment and is easily made. Cheng and Dijkstra (2007) reported that the continuous labelling method can be used for producing uniformly labelled litter for decomposition studies under a controlled environment.

2.3.3 Soil organic matter fractionation

Soil organic matter influences the physical, chemical and biological properties of soil. Soil organic matter is highly heterogeneous and theoretically can be divided into two or three pools, ranging from very active (labile) to stable (non-labile) (Comeau, 2012). The active pool contains easily decomposable compounds, which are labile fractions. The fractions in an active pool have a rapid decomposition rate, and can have a turnover time of less than a few decades (Campbell et al., 1967, Hsieh, 1992). The labile fractions are crucial to SOM status, because equilibrium between decay and renewal processes in this pool control nutrient availability (Wander et al., 1994). The stable pool, which stores the majority of SOM, and is physically protected in clay-humus composites. The fractions in this pool may have turnover times as long as several thousand years (Strosser, 2010).

The use of physical fractionations in separating SOM has increased steadily over the past two decades. The physical fractionation by size or density is generally accepted as a straightforward, reliable and reproducible method to separate SOM into different parts (Gregorich and Ellert, 1993). Fractionation methods isolate specific SOM constituents with different turnover rates and physically divide SOM into pools differing in composition and biological function (Christensen, 1992). Fractionation methods separate SOM into two components: (1) incompletely decomposed organic residues, i.e. the light fraction (LF); (2) mineral surface bonded or microaggregates contained organic matter, i.e. the heavy fraction (HF). The LF, regarded as highly labile and considered to be a major N source in various agricultural soils, is comprised largely of incompletely decomposed organic residues (Bcione et al., 1994). Compared to the LF, the HF is

more decomposed and humified, has a narrower C:N ratio and is generally more stable (Comeau, 2012). The HF is considered to be the principal N source in several forest soils (Bcione et al., 1994). The LF can be separated from soil using water or a denser solution, such as NaI at 1.7 g cm^{-3} (Gregorich et al., 2006). At the same time, it is possible to extract the very light fraction (VLF), which is mainly composed of fresh plant residues. The use of inorganic media (i.e. NaI) in density separation techniques alleviated concerns about toxicity and C contamination associated with the use of organic solvents (Gregorich et al., 1994)

3. CROPPING SEQUENCE EFFECT ON BIOLOGICAL N_2 FIXATION OF CHICKPEA, LENTIL AND FIELD PEA

3.1 Preface

Pulse crops included in diversified rotations have provided economic and environmental benefits due to their ability to fix atmospheric N_2 by forming symbiotic associations with N_2-fixing rhizobium. Therefore, the two major benefits of growing pulse crops are a reduced reliance on inorganic N fertilizers for the growth of the pulse crop, and the provision of N-rich residues for subsequent crops. However, the effect of different pulse crops on the performance of subsequent pulse crops in a rotation has not been thoroughly studied. This study used the enriched ^{15}N dilution method to examine BNF in three pulse crops (chickpea, lentil, and field pea), when grown in sequence with pulses, canola and wheat.

3.2 Abstract

This study examined cropping sequence effects on BNF for three pulse crops: chickpea, lentil and field pea. Soil cores from three different cropping sequences (chickpea-wheat, lentil-wheat, and pea-wheat) were sampled from Swift Current, SK. In addition, soil cores from two other cropping sequences (wheat-canola, canola-wheat) were extracted from a farmer's field in Central Butte, SK. Under controlled conditions, the three pulse crops were grown as the third crop in sequence and evaluated for BNF using the enriched ^{15}N isotope dilution technique. Nitrogen from BNF in seed, pod, leaf, and stem, and the productivity of each part were evaluated. By comparison, pea tended to have greater BNF than chickpea and lentil. Growing the same first year and third year pulse crop (chickpea-wheat-chickpea, lentil-wheat-lentil) produced more biomass and fixed more N_2 than growing a different pulse crop in year 1 and year 3 (pea-wheat-chickpea; pea-wheat-lentil). Growing pulse crops immediately after canola produced less biomass and had lower BNF compared to those grown with wheat as the preceding crop. Therefore, it is recommended that pulse crops not be grown following canola. This study provided information for pulse crop producers when choosing pulse crops for rotation to capitalize on BNF in specific soils.

3.3 Introduction

Traditional continuous non-legume cropping systems rely heavily on N fertilizers. Indeed, N fertilizer is one of the major economic costs of continuous cropping systems in western Canada (Beckie and Brandt, 1997). Over the past decade, N fertilizer prices have increased dramatically due to rising oil prices and to increased demand for N. In addition, it is estimated that production and application of N fertilizers produced 57% to 65% of the total CO_2-equivalent emissions from Canadian prairie agricultural systems making application of N fertilizer as a major factor associated with climate change (Gan et al., 2011b).

Pulse crops included in rotation systems decreases reliance on inorganic N fertilizers because of their ability to form symbiotic associations with N_2-fixing *Rhizobium* bacteria (Crews and Peoples, 2004). Amounts of N_2 fixed by pulse crops vary considerably. Chickpea, lentil and field pea can be ranked according to estimated ability to fix N_2: field pea>chickpea>lentil (Walley et al., 2007). The determination of the amount of N_2 fixed is closely linked to %Ndfa,

which is extremely variable among different pulse crops (Evans et al., 2001; Walley et al., 2007). The %Ndfa ranges from 0% to 81%, and is partly dependent on the effectiveness of symbiotic associations (Walley, 2007; Soon and Lupwayi, 2008; Peoples et al., 2009).

Crop rotation sequence also can influence BNF (Slattery et al., 2001; Knight, 2012). For growing pulse crops, crop rotations have an impact on external environmental conditions, such as insect and weed population (Corre-Hellou and Crozat, 2005; Strydhorst et al., 2008), soil nutrient availability (Peoples et al., 2001; Walley et al., 2007; Kellman, 2008; Mohammadi, 2012) and soil microorganisms (Augus et al., 1994; Gan et al., 2002; Al-falih, 2002). Diversifying crop species in rotations decreases disease and diminishes weed populations, improves the productivity of pulse crops and thereby affecting the amount of N_2 fixed (Krupinsky et al., 2002; Seymour et al. 2012). In addition, crop residue inputs to soil may affect the BNF due to the change in soil nutrient levels (Jensen 1996; Lupwayi and Kennedy, 2007). For example, pulse crop residues confer N benefits to subsequent crops through the release of N by residue mineralization (Jensen 1996). However, increased initial soil N levels may inhibit BNF in growing pulse crops. Furthermore, including different crops in rotation may change the microbial populations in soil (Gan et al., 2002; Al-falih, 2002). A greater diversity of microorganisms can be found in soils where diverse crop species are grown (Lupwayi et al., 1998; Wieland et al., 2001). This increased microbial biodiversity in the rotation may enable greater adaptability to environmental and biotic fluctuations that affect BNF (Knight, 2012).

The objectives of this study were to: (1) to compare BNF in pea, lentil and chickpea grown in the same cropping-history (pea-wheat-pulse; wheat-canola-pulse; canola-wheat-pulse); (2) to determine if the previous pulse crop in rotation with wheat (pulse-wheat-pulse) affects BNF in the subsequent pulse crop; and (3) to examine whether there is a difference between cereal (wheat) and oilseed (canola) in a rotation on the subsequent BNF of chickpea, lentil and field pea.

3.4 Materials and methods

3.4.1 Soil collection

Soil cores (20 cm dia. by 30 cm depth) were collected from Agriculture and Agri-Food Canada's Semiarid Prairie Agriculture Research Centre located at Swift Current, SK (AAFC-SPARC) and from a farmer's field located at Central Butte, SK. Soil cores at AAFC-SPARC were extracted from three crop rotations (pea-wheat, lentil-wheat and chickpea-wheat). At Central Butte, soil cores were extracted from two crop rotations (canola-wheat and wheat-canola). Aluminum cylinders (20-cm dia. by 30-cm depth) were pushed into the soil using a truck mounted hydraulic punch and carefully withdrawn to keep soil structure intact. The soil cores with the crop residues were stored frozen over the winter period and then stored in a greenhouse until seeding six months after the cores were removed from the field. The soils from Swift Current and Central Butte are classified as an Orthic Brown Chernozem and as Orthic Rego Brown Chernozem, respectively.

3.4.2 Experimental design

This study was carried out in a greenhouse. Experiments were conducted with three N_2-fixing pulse crop species: chickpea, lentil and field pea following different rotation sequences and conducted in two separate experiments (Table 3.1). Soil cores were watered for one week before seeding to stabilize the microbial population in the soil. At the time of seeding, lentils and field peas were inoculated with a peat- based inoculant containing *Rhizobium leguminosarum* bv. *viciae* (Nodulator®XL, Becker Underwood Inc., Saskatoon, SK), whereas chickpeas were inoculated with a self-adhering peat-based inoculant containing *Bradyrhizobium sp. (Cicer)* (Nodulator®, Becker Underwood Inc., Saskatoon, SK). Inoculants were applied according to the manufacturer's recommendation. Eight seeds for each pulse crops were planted per core. Soil cores were watered thoroughly every two days. After emergence, plants density was reduced to four per core. At this time, the soil cores were moved to a bench in the greenhouse for $^{13}CO_2$ labelling. The bench was fitted with an aerial tent that inhibited access to the soil cores. The details of the ^{13}C labelling are discussed in Chapter 4. Because of the impeded access to the soil cores in a number of cores, plants emerging after cores were moved were not removed, resulting in different plant densities of harvest (Table 3.1). Plants were grown at an average day time

temperature of 22 °C in the greenhouse. Treatments (rotation sequences) were replicated three times in Experiment 1 and four times in Experiment 2. Numbers of replicates in Experiment 1 were limited by the number of cores which could be removed from the field without comprising ongoing field experiments.

Table 3.1. Field cropping history and crops grown in two greenhouse experiments. Intact soil cores were extracted from field study at AAFC-Swift Current and field at Central Butte.

Experiment	Field Site	Cropping Sequence			Plant Density at Harvest
		Year 1 Field crop	Year 2 Field crop	Year 3 Greenhouse crop	
1	AAFC	Chickpea	Wheat	Chickpea	4
1	AAFC	Lentil	Wheat	Lentil	4
1	AAFC	Field Pea	Wheat	Field pea	4
1	AAFC	Field pea	Wheat	Chickpea	4
1	AAFC	Field pea	Wheat	Lentil	3
2	Central Butte	Wheat	Canola	Chickpea	5
2	Central Butte	Wheat	Canola	Lentil	5
2	Central Butte	Wheat	Canola	Field pea	3
2	Central Butte	Canola	Wheat	Chickpea	5
2	Central Butte	Canola	Wheat	Lentil	5
2	Central Butte	Canola	Wheat	Field pea	4

3.4.3 ^{15}N labelling procedure and plant sample preparation

Biological N_2 fixation was estimated using the ^{15}N enriched dilution technique (Hardarson and Danso, 1990). Four weeks after seeding, ^{15}N-enriched NH_4- NO_3 solution (10 atom% excess) was applied to the soil surface of each core at a rate equivalent to 5 kg ha^{-1}. Wheat was included as the non-N_2 fixing crop for BNF measurements in both experiments. Fifteen weeks after emergence, all shoot materials were harvested at the soil level. Shoots were dried at 40 °C to stable dry weight then separated into seeds, pods, leaves and stems. Plant parts were weighed for dry biomass, ground with a Wiley mill, and then finely ground using a ball mill grinder. The ground plant parts were analyzed for total N concentration (%) and atom%^{15}N with a Costech Elemental Combustion System coupled to a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc). A second set of plants was established in a separate greenhouse chamber and did not receive ^{15}N-enriched NH_4- NO_3. These natural abundance plants were treated the same as plants in the ^{15}N enrichment experiment. The natural abundance level of ^{15}N in each plant part was used to calculate atom ^{15}N excess in the ^{15}N enriched plant part (Eq. 3.1).

$$\text{Atom\%}^{15}\text{N}_{excess} \text{ in plant part} = \text{atom\%}^{15}\text{N}_{\text{ labelled plant part}} - \text{atom\%}^{15}\text{N}_{\text{ non-labelled plant part}}$$

(Eq. 3.1)

Percentage of N derived from atmosphere (%Ndfa) per plant part was calculated according to Hardarson and Danso (1990):

$$\%\text{Ndfa in plant part} = [1- \frac{\text{atom \%}^{15}\text{N excess in plant part of fixing crop}}{\text{atom \%}^{15}\text{N excess in plant part of non-fixing crop}}] \times 100$$

(Eq. 3.2)

$$\text{Total N in plant part of fixing crop} = \frac{(\%\text{N in each plant part}) \times \text{dry biomass of each plant part}}{100}$$

(Eq. 3.3)

The amount of the BNF per plant part was determined by the formula (Hardarson and Danso 1990) below:

$$\text{BNF in plant part} = \frac{\%\ \%\text{Ndfa in plant part} \times \text{total N in plant part of fixing crop}}{100}$$

(Eq. 3.4)

The amount of BNF in straw is the sum of BNF in pods, stems and leaves.

The amount of N acquired from the soil in each plant part was calculated as:

$$\text{N acquired from soil in plant part} = \text{total N in plant part} - \text{BNF in plant part}$$

(Eq. 3.5)

3.4.4 Statistical analysis

All data were tested for normality using Shapiro-Wilk test (P>0.05) and homogeneity of variance using Bartlett's test (P>0.05). Data from Experiment 1 were analyzed using a one-way analysis of variance (ANOVA), with rotation sequence as the main factor. In addition, means

comparisons were made using the Tukey's Honestly Significant Different (HSD) test. Furthermore, contrasts were performed to determine the difference between different rotation sequences, and different pulse crops. Data from Experiment 2 were analyzed using a 2-way ANOVA with two levels of the preceding crop sequence (wheat-canola and canola-wheat) and three levels of the experimental crop (chickpea, lentil and field pea). Effects were declared significant at $P<0.05$. Statistical analyses were performed using the statistical program R Foundation for Statistical Computing version 2.10.0 (R Development Core Team, 2010).

3.5 Results

3.5.1 Influence of the preceding pulse crop in sequence (Experiment 1)

The effect of rotation on biomass differed, depending on the crop (Table 3.2). Lentil tended to produce the least total biomass (seed, pod, stem and leaf) and chickpea tended to produce the greatest total biomass among crops, but differences were not statistically significant. The seed biomass produced by lentil was the least and pea was the most among crops. Comparing the impact of previous pulses in a rotation on crop biomass, growing the same first and third year crop in rotation (chickpea-wheat-chickpea and lentil-wheat-lentil) produced more total aboveground biomass than growing different first and third year crops in rotation (pea-wheat-chickpea and pea-wheat-lentil) (P=0.046) (Table 3.3).

The percentage of N derived from BNF in lentil was affected by the rotation sequence (Table 3.2). Except for %Ndfa in the stem, %Ndfa in all other plant parts was greater for lentil grown in the lentil-wheat-lentil rotation than lentil grown in the pea-wheat-lentil rotation. Overall, %Ndfa in chickpea was not affected by rotation sequence.

Compared to chickpea and lentil, pea fixed the greatest amount of N_2 in the plants (51.7 mg per plant) among crops (Table 3.2 and Table 3.3). Lentil had the least amount of N_2 fixed in the aboveground shoots (seed, pod, stem and leaf) compared to chickpea and pea. Pea fixed the most N_2 in aboveground shoots among crops. Lentil in the rotation lentil-wheat-lentil fixed a significantly greater amount of N_2 in the shoot than lentil grown in rotation pea-wheat-lentil.

The N in plant parts originating from either BNF or from the soil, varied widely among the crops. The percentage of fixed N_2 to total N in shoots of chickpea, lentil and pea was 41%, 16% and 43%, respectively (calculated as the amount of fixed N_2 divided by the amount of total N). A

lower amount of N was obtained from BNF in lentil than in chickpea and pea. This indicated that lentil acquired more of its N from the soil compared to that of chickpea and pea. In terms of cropping sequence effect, no significant difference was found in total N amount and N from soil in aboveground shoots among all rotations (Table 3.4).

3.5.2 Influence of the preceding non-pulse crop in sequence (Experiment 2)

Seed biomass production was very low in chickpea and lentil (Table 3.5) and this finding was probably due to differences in maturity among the three pulse crops. By comparison, pea had the greatest total aboveground shoot biomass (seed, pod, stem and leaf) among crops. Total biomass was affected by the crop sequence with pulse crops immediately preceded by canola always producing less biomass than a pulse crop immediately preceded by wheat.

Overall, the %Ndfa of chickpea parts was greater than that of lentil and field pea (Table 3.5). The %Ndfa in pods of chickpea and pea was greater when the pulse cops were grown after canola than wheat. The %Ndfa of lentil was not affected by preceding crop.

Growing pulse crops and preceding crops significantly affected the amount of N_2 fixed in each plant part. The greatest amount of fixed N_2 in shoots (seed and straw) occurred in pea (average 3.11 and 3.68 mg per plant), the least amount of N_2 fixed in shoots was in lentil (2.39 and 3.18 mg per plant) (Table 3.5). The preceding crop also affected BNF. Wheat grown prior to a pulse crop resulted in a greater amount of N_2 fixed by pulse crop than by the pulse crop following canola.

The source of N in the parts varied among the actively growing pulse crop and with the different preceding crops (Table 3.6). Among the crops grown in the cores, chickpea obtained the most N from BNF. The amount of N_2 fixed of total aboveground N in chickpea, lentil and pea was approximately 71%, 51% and 51% of total N, respectively. Comparing the preceding crop effect, pulse crops following wheat had greater amounts of total N, amount of N_2 fixed and N acquired from soil in all plant parts compared to the same crops following canola.

Table 3.2. Biomass produced, %N derived from fixation (%Ndfa) and amount of fixed N_2 per plant in plant parts of chickpea, lentil and field pea in five rotation sequences.

Rotation sequence	Seed	Pod	Stem	Leaf	Straw†	Seed + Straw
			Biomass (g plant^{-1})			
CP-W-**CP**‡	1.69 (0.87)a§¶	0.86 (0.35)a	2.01 (0.32)a	1.67 (0.47)a	4.55 (0.73)a	6.24 (0.98)a
L-W-**L**	0.22 (0.10)b	0.25 (0.09)b	2.68 (0.32)a	2.03 (0.35)a	4.96 (0.76)a	5.19 (0.74)a
P-W-**P**	2.58 (0.40)a	0.64(0.16)a	0.59 (0.14)b	1.17 (0.19)a	2.40 (0.46)b	4.98 (0.85)a
P-W-**CP**	1.52 (0.79)a	0.69 (0.04)a	2.28 (0.47)a	1.61 (0.34)a	4.58 (0.78)a	6.10 (1.20)a
P-W-**L**	0.12 (0.05)b	0.16 (0.06)b	1.97 (0.34)a	1.96 (0.36)a	4.09 (0.67)a	4.02 (0.73)b
P value	**<0.001**	**0.003**	**<0.001**	0.088	**0.008**	**0.009**
			%Ndfa			
CP-W-**CP**	43.3 (3.1)a	40.9 (0.3)a	40.7 (0.4)a	15.9 (3.6)b	-	-
L-W-**L**	47.2 (4.8)a	41.1 (1.1)a	20.0 (1.2)b	12.6 (3.1)b	-	-
P-W-**P**	45.2 (3.3)a	17.5 (2.8)b	40.4 (6.3)a	33.7 (3.9)a	-	-
P-W-**CP**	47.7 (4.9)a	42.5 (1.8)a	38.8 (9.9)a	21.6 (18.2)a	-	-
P-W-**L**	31.8 (3.6)b	23.1 (7.2)b	21.1 (10.9)b	5.1 (0.7)c	-	-
P value	**0.004**	**0.027**	**0.011**	**<0.001**	-	-
			Fixed N_2 (mg plant^{-1})			
CP-W-**CP**	21.2 (9.2)b	4.3 (1.1)a	7.7 (3.3)a	2.3 (2.3)b	16.1 (4.9)a	38.1 (4.9)b
L-W-**L**	6.9 (2.4)c	2.9 (1.2)a	10.9 (1.4)a	8.9 (1.3)a	22.9 (2.8)a	29.8 (2.8)b
P-W-**P**	44.3 (5.4)a	1.1 (0.3)b	1.9 (0.6)b	4.3 (0.7)b	7.4 (1.1)b	51.7 (6.4)a
P-W-**CP**	24.5 (9.7)b	3.1 (0.7)a	8.1 (3.2)a	7.7 (0.9)a	19.0 (3.8)a	42.4 (12.4)b
P-W-**L**	1.4 (0.7)c	0.7 (0.1)b	4.4 (1.5)ab	2.1 (0.3)b	7.1 (1.2)b	8.5 (0.8)c
P value	**<0.001**	**0.001**	**0.005**	**<0.001**	**<0.001**	**<0.001**

† Straw includes pod, stem and leaf.
‡ CP: chickpea, W: wheat, L: lentil and P: field pea.
§ Data of seed biomass and %Ndfa in pod was log transformed for ANOVA.
¶ Same letter following mean ± standard errors (n=3) indicate no significant difference within each plant part in rotation (P<0.05) according to Tukey's HSD test.

Table 3.3. Summary of *P*-values from contrasts.

Contrast	Treatments	*P-value*	
		Total biomass†	Total amount of fixed N_2
Same 1st and 3rd crop vs different 1st and 3rd crop	CP-W-CP and L-W-L vs P-W-CP and P-W-L‡	**0.046**	**0.002**
CP vs L as 3rd crop	CP-W-CP and P-W-CP vs L-W-L and P-W-L	**0.044**	**<0.001**
CP vs P as 3rd crop	CP-W-CP and P-W-CP vs P-W-P	0.205	**0.002**
L vs P as 3rd crop	L-W-L and P-W-L vs P-W-P	0.612	**<0.001**

† Total includes seed, pod, stem and leaf.
‡ CP: chickpea, W: wheat, L: lentil and P: field pea.

Table 3.4. Total N mass, total fixed N_2 and total N from soil in plant parts of chickpea, lentil and field pea in different rotation sequence.

Rotation sequence	Total N (mg per plant)			Fixed N_2 (mg per plant)			N from soil (mg per plant)		
	Seed	Straw†	Seed + Straw	Seed	Straw	Seed + Straw	Seed‡	Straw‡	Seed + Straw
CP-W-**CP**§	54.0 (32.2) a¶	52.0 (5.8) b	106.0 (26.8) ab	21.2 (9.2) b	16.1 (4.9) a	38.1 (4.9) a	32.0 (23.1) a	35.9 (2.9) c	67.9 (22.9) b
L-W-**L**	14.6 (4.4) c	135.9 (26.4) a	150.5 (26.8) a	7.0 (2.4) c	22.9 (2.8) a	29.8 (2.8) a	7.6 (2.1) b	113.1 (24.3) a	120.7 (24.0) a
P-W-**P**	97.9 (7.1) a	24.0 (2.2) c	121.9 (8.9) ab	44.3 (5.4) a	7.4 (1.1) b	51.7 (6.5) a	53.6 (4.2) a	16.6 (1.2) d	70.2 (4.6) b
P-W-**CP**	50.4 (24.4) a	51.1 (7.9) b	101.5 (25.5) ab	24.5 (9.7) b	19.0 (3.8) a	42.4 (12.4) a	30.8 (18.1) a	34.9 (5.9) c	65.7 (18.9) b
P-W-**L**	4.4 (2.2) b	66.9 (12.7) b	71.3 (14.7) b	1.4 (0.7) c	7.1 (1.2) b	8.5 (0.8) b	3.0 (1.6) b	59.8 (13.5) b	62.8 (14.8) b
P value	**<0.001**	**<0.001**	**0.015**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.015**

† Straw includes pod, stem and leaf.
‡ Data was run transformated for ANOVA.
§ CP: chickpea, W: wheat, L: lentil and P: field pea.
¶ Same letter following mean ± standard errors (n=3) indicate no significant difference within each plant part in rotation ($P<0.05$) according to Tukey's HSD test.

Table 3.5. Biomass produced, %Ndfa and amount of fixed N_2 in plant parts of chickpea, lentil and field pea following canola and wheat.

Rotation sequence	Seed†	Pod	Stem	Leaf	Straw‡	Seed +Straw
			Biomass (g plant^{-1})			
W-CNL-**CP**§	0.070 (0.012) ¶	0.017 (0.012)	1.72 (0.16)	1.37 (0.02)	3.10 (0.16)	3.16 (0.15)
CNL-W-**CP**	0.083 (0.017)	0.022 (0.009)	1.80 (0.22)	1.44 (0.18)	3.25 (0.37)	3.31 (0.38)
W-CNL-**L**	0.10 (0.07)	0.11 (0.04)	1.49 (0.10)	0.69 (0.16)	2.28 (0.23)	2.39 (0.16)
CNL-W-**L**	0.43 (0.17)	0.23 (0.01)	1.72 (0.70)	0.79 (0.12)	2.74 (0.37)	3.18 (0.29)
W-CNL-**P**	1.22 (0.39)	0.29 (0.09)	0.74 (0.15)	0.85 (0.16)	1.88 (0.34)	3.11 (0.71)
CNL-W-**P**	1.42 (0.19)	0.27 (0.05)	1.11 (0.28)	0.87 (0.27)	2.25 (0.02)	3.68 (0.21)
Summary of P values from ANOVA for biomass produced by each part for three crops following wheat and canola						
Crop	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.022**
Preceding	**<0.001**	0.074	**0.021**	0.329	**0.017**	**0.006**
Crop × Preceding	**0.008**	**0.037**	0.397	0.867	0.571	0.277
			%Ndfa			
W-CNL-**CP**	77.2 (2.5)	44.8 (0.5)	72.0 (3.00)	72.5 (2.9)	-	-
CNL-W-**CP**	81.0 (5.0)	74.8 (2.8)	68.7 (2.5)	70.0 (4.5)	-	-
W-CNL-**L**	52.3 (8.0)	64.0 (6.1)	47.1 (4.9)	51.2 (7.9)	-	-
CNL-W-**L**	57.5 (7.0)	64.9 (3.7)	42.6 (6.9)	50.1 (5.9)	-	-
W-CNL-**P**	54.7 (7.3)	47.2 (10.5)	45.5 (10.4)	48.4 (10.0)	-	-
CNL-W-**P**	52.6 (1.5)	58.9 (1.3)	42.8 (7.8)	45.5 (3.9)	-	-
Summary of P-values from ANOVA for %Ndfa in each part for three crops following wheat and canola						
Crop	**<0.001**	**0.006**	**<0.001**	**<0.001**	-	-
Preceding	0.410	**<0.001**	0.231	0.482	-	-
Crop × Preceding	0.523	**0.002**	0.964	0.966	-	-
			Fixed N_2 (mg plant^{-1})			
W-CNL-**CP**	1.9 (0.3)	0.3 (0.0)	14.3 (2.4)	30.2 (2.2)	44.7 (4.0)	46.1 (3.3)
CNL-W-**CP**	2.4 (0.5)	0.2 (0.1)	21.1 (3.0)	27.3 (3.8)	48.6 (4.8)	50.4 (3.8)
W-CNL-**L**	3.1 (1.3)	1.9 (0.5)	9.9 (2.3)	12.6 (4.8)	24.3 (6.4)	27.4 (6.5)
CNL-W-**L**	10.4 (3.5)	3.5 (0.4)	9.3 (2.7)	11.9 (2.5)	24.7 (5.5)	35.2 (3.8)
W-CNL-**P**	30.1 (12.6)	2.0 (0.5)	5.3 (1.2)	11.8 (2.8)	19.1 (3.9)	49.2 (9.5)
CNL-W-**P**	33.1 (4.1)	2.3 (0.2)	8.4 (4.1)	10.8 (1.1)	21.5 (3.0)	54.6 (3.4)
Summary of P-values from ANOVA for fixed N amount in each part for three crops following wheat and canola						
Crop	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Preceding	**<0.001**	**<0.001**	**0.013**	0.509	0.331	**0.024**
Crop × Preceding	**0.016**	**0.003**	**0.043**	0.930	0.846	0.832

† Data of seed biomass; data of fixed N_2 of seed, straw and leaf was log transformed for ANOVA analysis.

‡ Straw includes pod, stem and leaf.

§ W: wheat, CNL: canola, CP: chickpea, L: lentil and P: field pea.

¶ Values are means (n=4) ± standard errors

Table 3.6. Total N mass, total fixed N and total N from soil in plant parts of chickpea, lentil and field pea in following canola and wheat.

Rotation sequence	Total N (mg per plant)			Fixed N_2 (mg per plant)			N from soil (mg per plant)		
	Seed†	Straw‡	Seed + Straw†	Seed	Straw†	Seed + Straw	Seed†	Straw	Seed + Straw
W-CNL-**CP** §	2.4 (0.4) ¶	62.0 (6.1)	63.8 (5.1)	1.9 (0.3)	44.7 (3.9)	46.1 (3.3)	0.42 (0.30)	17.3 (2.8)	17.7 (2.5)
CNL-W-**CP**	2.3 (1.6)	70.1 (7.6)	72.3 (6.7)	2.4 (0.5)	48.6 (4.8)	50..4 (3.8)	0.43 (0.36)	21.4 (4.2)	21.9 (4.0)
W-CNL-**L**	5.8 (2.0)	47.8 (7.4)	53.6 (7.7)	3.1 (1.3)	24.3 (6.4)	27.4 (6.5)	2.6 (0.7)	23.5 (1.2)	26.2 (1.3)
CNL-W-**L**	18.3 (6.6)	50.6 (5.3)	68.8 (3.3)	10.4 (3.5)	24.7 (5.5)	35.2 (3.8)	7.9 (3.5)	25.8 (1.4)	33.7 (4.0)
W-CNL-**P**	54.6 (18.9)	40.5 (3.3)	95.2 (21.2)	30.1 (12.6)	19.1 (3.9)	49.2 (9.5)	24.6 (8.2)	21.4 (5.4)	50.0 (13.5)
CNL-W-**P**	62.8 (6.1)	46.8 (2.4)	109.6 (3.9)	33.1 (4.1)	21.5 (3.0)	54.6 (3.4)	29.7 (2.2)	25.2 (1.3)	55.0 (2.7)
Summary of P values from ANOVA for total N, fixed N and N from soil for three crops following wheat and canola									
Crop	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.782
Preceding	**0.030**	**0.033**	**0.002**	**0.001**	0.331	**0.024**	**0.010**	**0.007**	**0.006**
Crop × Preceding	**0.056**	0.652	0.545	**0.016**	0.846	0.832	**0.015**	0.688	0.490

† Data was log transformed for ANOVA.
‡ Straw includes pod, stem and leaf.
§ W: wheat, CNL: canola, CP: chickpea, L: lentil and P: field pea.
¶ Values are means (n=4) ± standard errors.

3.6 Discussion

Gan et al. (2010) and Herridge et al. (2008) report that global averages for BNF by chickpea, lentil and pea are 58, 51, 86 kg N ha^{-1}, respectively. Based on the planting densities of chickpea of 40 plants m^{-2}, lentil of 120 plants m^{-2} and pea of 80 plants m^{-2}, the extrapolated amounts of N_2 fixed in total shoot in Experiment 1 were 16, 21 and 43 kg N ha^{-1} for chickpea, lentil and pea. For Experiment 2, chickpea, lentil and pea fixed approximately 20, 39, 43 kg N ha^{-1}, respectively. Similar to Gan et al. (2010) and Herridge et al. (2008), pea fixed the most N_2 among pulse crops grown in common rotation sequence, following by chickpea. The pulse crops matured earlier harvest in my study compared to pulse crops in commercial production fields. One possible factor was planting densities; i.e. the density of pulse crops in the current study was 125 plants m^{-2}. The increase in plant density result in increased competition for resources for growth compared to plants grown in the field.

Cropping the same pulse crop in years 1 and 3 in a three-year rotation (e.g. chickpea-wheat-chickpea and lentil-wheat-lentil) was better than cropping different pulse crops grown in a three-year rotation with wheat (pea-wheat-chickpea and pea-wheat-lentil) (Table 3.2 and Table 3.3). Lentil in a rotation that included the same pulse in a three-year rotation (lentil-wheat-lentil) produced approximately 40% more biomass and obtained 2.5 times more N from BNF than lentil in rotation with a different pulse (pea-wheat-lentil). Similarly, chickpea in the rotation sequence chickpea-wheat-chickpea tended to produce more biomass compared to chickpea in the pea-wheat-chickpea sequence. One possibility for this difference in the amount of fixed N_2 may be due to a difference in microbial communities. The soil microbial communities beneficial to growth of a specific pulse crop may be conserved in the soil and benefit the same pulse crop later in the rotation compared to grow a different pulse crop. In addition, soils with a history of pulses may contain residual rhizobia that can produce N_2 fixing nodules (Government of Saskatchewan, 2007). The rhizobia surviving in the soil may be more efficient for the same pulse crop, because each pulse crop requires specific bacteria species for nodulation (Government of Saskatchewan, 2007). This is in contrast to previous studies that credit developing diversity of crops in a rotation with increasing productivity and BNF (Matus et al., 1997; Hardson, 2003). Marais et al. (2012) stated that microbial communities under wheat monoculture conditions would be significantly different from microbial communities where wheat is in rotation with legumes. The

increased microbial biodiversity in a rotation makes it more flexible in responding to environmental changes that affect productivity and BNF (Lupwayi 1998; Wieland et al., 2001). However, the "flexibility" may not expressed because of more consistent conditions in the greenhouse.

All three pulse crops obtained a greater amount of N_2 from BNF when following wheat, compared to when following canola (Table 3.5). As well, greater aboveground biomass was produced by pulse crops with wheat as the preceding crop than that produced with canola as the preceding crop. However, in a previous field study, pulse crops were not significantly influenced by a preceding canola crop (Knight, 2012). This is likely due to differences in the soils in which the experiments were performed. The soil cores collected for my study were Brown soils, but the soil zone where Knight's study (2012) was performed was the Dark Brown soil. The Dark Brown soil had higher organic matter which may buffer against the adverse effects of the canola residue. The effects of phytotoxic compounds from canola residue have been reported (Wanniarachichi and Voroney, 1997). Phenolic compounds are the major class of phytotoxic compounds that decrease the growth rate of roots and shoots of subsequent plants. Furthermore, growing canola in rotation may reduce beneficial-organism populations such as rhizobia and arbuscular mycorrhizal fungi (AMF) in the subsequent crops. Because these microorganisms do not colonize canola roots, the populations of these microorganism will decrease after a canola crop (Krupinsky et al., 2006). Arbuscular mycorrhizal fungi reportedly increased chickpea yields due to increased phosphorus uptake (Erman et al., 2011; Ortas, 2012; Reen 2014). In addition to reducing beneficial microorganism, growing canola in rotation may result in poorer soil quality than that obtained by growing a cereal crop in rotation (Bourgeois and Entz, 1996; Wanniarachchi and Voroney, 1997). Canola produces less crop residue and decays more quickly than a cereal crop (Canola Council of Canada, 2014), which may lead to a less protected surface soil with a high possibility of soil erosion and loss. Therefore, pulse plant growth may be affected by soil quality in such situations.

Pulse crops planted in Experiment 2 obtained a higher proportion of N via BNF than crops grown in Experiment 1. For chickpea, lentil and pea in Experiment 1, the average proportions of N derived from BNF to total N in shoots were approximately 41%, 16% and 43%, respectively. In Experiment 2, N acquired via BNF to total N in chickpea, lentil and pea accounted for nearly

70%, 51% and 51% of total N in shoots, respectively. The enhanced BNF in Experiment 2 was likely associated with plant density and initial soil N levels. In general, after soil N is depleted, N acquired from BNF becomes the main N source for grown crops. Consequently, a high starter N level may delay the occurrence of BNF. The higher plant density of chickpea and lentil in Experiment 2 than Experiment 1 may have resulted in higher intra-specific competition for soil N, resulting in an increase in the proportion of N in plants acquired from BNF (Danso et al., 1987). However, pea was planted in the same density in both experiments but the pea grown in Experiment 2 still had a higher proportion of N obtained from BNF to total N than pea grown in Experiment 1. In the current study, potentially higher start N levels may have occurred in soil cores for Experiment 1 than for Experiment 2, possibly reflecting to the history of the crop rotation. The first-year crop in the three-year rotation for Experiment 1 was a pulse crop, but for Experiment 2 it was a cereal or oilseed crop. Pulse crop residue remaining after harvesting have narrower C:N ratios than cereals and oilseeds (Lupwayi and Kennedy, 2007). The narrower C:N ratios in pulse crop residue makes it suitable for N mineralization, enhancing available inorganic N in the soil for subsequent crops (Jenson, 1994; Steven, 1996). Higher inorganic N levels from the mineralization of the previous pulse residues have decreased BNF thus increasing reliance in soil inorganic N compared to pulse crops preceded by wheat and canola.

3.7 Conclusion

With the same history of rotation, pea tended to have the highest productivity and fixed a greater amount of N_2 compared to chickpea and lentil. The same pulse crop included in a three-year rotation alternating with wheat (chickpea-wheat-chickpea; lentil-wheat-lentil) produced more biomass and fixed more N_2 than two different pulse crops in a three-year rotation (pea-wheat-chickpea; pea-wheat-lentil). In addition, canola grown as the preceding crop had an adverse effect on productivity and BNF of the following pulse crops. Pulse crops immediately following canola produced less biomass and fixed less N_2 than pulse crops with wheat as a preceding crop. Compared to pulse crops grown in Experiment 1, a markedly higher %Ndfa occurred for pulse crops in Experiment 2. Due to the large difference in %Ndfa, pulse crops in Experiment 1 fixed less N_2 than crops in Experiment 2.

4. USE OF CONTINUOUS LABELLING WITH DEPLETED $^{13}CO_2$ TO FOLLOW THE FATE OF ^{13}C TO SOIL FROM CHICKPEA, LENTIL AND FIELD PEA.

4.1 Preface

Growing pulse crops in rotation provides C sequestration benefits by improving soil C storage. The C inputs from pulse crops to soil are from root rhizodeposits during growth and root and straw residues after harvest. This chapter estimates the C inputs of chickpea, lentil and pea to soil as they are affected by cropping sequence. The cropping effect of previous crops (wheat, canola, or pulse) on actively growing pulse crops on soil C pools has not been thoroughly studied. Therefore, this chapter uses a continuous labelling with depleted $^{13}CO_2$ method to estimate ^{13}C inputs of pulse crop rhizodeposits (chickpea, lentil and field pea) to various soil fractions as influenced by cropping sequence. In addition, the efficiency of this method for tracking ^{13}C movement from plant to soil is discussed in this chapter.

4.2 Abstract

Increasing C sequestration in agricultural soils improves soil fertility, reduces greenhouse gas emission and increases agronomic productivity. Pulse crop residues have been shown to input more C into soil relative to canola and wheat, thereby providing C sequestration benefits. A method in which CO_2 depleted in ^{13}C was evaluated for estimating C inputs from three of pulse crops, chickpea, lentil and pea. The objectives of this study were to: 1) evaluate the effectiveness of a continuous labelling with depleted $^{13}CO_2$ method to track ^{13}C movement from the growing chickpea, lentil and pea to soil during a single growing season, and 2) examine soil C pools as influenced by the growing pulse crop (chickpea, lentil and pea) and the previous crop (oilseed, cereal and pulse) in rotation. Soil cores from three crop rotations (chickpea-wheat, lentil-wheat and pea-wheat) were extracted from Swift Current, SK, soil cores also were extracted from two rotations (canola-wheat, wheat-canola) in a farmer's field in Central Butte, SK. Under controlled conditions, plants were exposed to CO_2 depleted in ^{13}C throughout the growing season until harvest. Carbon dioxide depleted in ^{13}C was derived from propane combustion. The distributions of the ^{13}C throughout the plant parts (seed, pod, leaf, stem and root) and SOM fractions (VLF, LF and HF) were determined. All plant parts (seed, pod, stem, leaf and root) were depleted in ^{13}C in this study. The movement of ^{13}C from plants to soil C pools was obtained, but the amount of ^{13}C transferred was insufficient for accurate calculations. Therefore, cropping sequence could not be evaluated using this continuous labelling with depleted $^{13}CO_2$ method. However, the ^{13}C-depletion in the soil fractions was obtained only in the VLF when plants were grown in depleted $^{13}CO_2$ conditions during that growing season. Compared to lentil and pea, chickpea contributed a higher amount of C to soil. As well, most of the new derived soil C from chickpea was found in the VLF. The newly derived ^{13}C did not significantly affect the LF and HF pools. It will be helpful to apply residue of labelled plant to follow C examine soil C pools following a growing season.

4.3 Introduction

An awareness of the deleterious effects of global climate change has increased as levels of atmospheric carbon dioxide (CO_2) have increased. One proposed method to reduce atmospheric CO_2 is to increase soil C storage through C sequestration. Soil organic C, the major component of SOM, is considered as an important part of soil for its contribution to soil productivity (Fenton et al., 1999; Al-Kaisi 2001). The SOM includes the organic constituents in the soil, including parts

from residual plant and animal material, products produced as these materials decompose, and the soil microbial biomass (Milne, 2012). Soil organic carbon has received considerable attention for its role in C fluxes and C sequestration in recent years, particularly in agricultural soils (Sartori et al., 2006; Chiti et al., 2012; Gosling et al., 2013). The SOC status in agricultural soils is dynamic, changing as organic matter is input to soil and SOM is decomposed (Paustian et al., 2000; Follett, 2001).

Different plant residue types affect soil C status (Gan et al., 2002; Halvorson et al., 2002; Sainju et al., 2008; Lenssen et al., 2007; Al-Kaisi 2001; Shrestha et al., 2013). Including pulses in a crop rotations may improve soil C status (Al-Kaisi 2001). Compared to traditional continuous wheat cropping, including pulse crops in rotations enables the soil environment to support large and more diverse populations of soil microbial organisms through rhizodeposition during crop growth (Lupwayi et al., 1998; Gan et al., 2002; Jensen and Hauggaard-Nielsen, 2003), thereby stimulating reside decomposition. This is likely associated with greater amounts and different types of amino acids in pulse crop root exudates that feed more soil microorganisms than non-legumes (Gan et al., 2002; Jensen and Hauggaard -Nielsen, 2003). Furthermore, the quality of a crop residue is a key factor impacting the amount of residue C stabilized as SOC (Lemke et al., 2007). Residues with wide C:N ratios are resistant to decomposition by soil microorganisms, in turn leading to low mineralization of these residues (Brady and Weil, 2008). Compared to wheat and canola residues, the generally narrower C:N ratio of pulse crop residue contributes to higher decomposition and mineralization rates of residues and faster conversion of residue C to SOC (Lemke et al., 2007).

Different fractions of SOM can provide information about soil quality and soil function. According to the model proposed by Comeau (2012), VLF SOM is derived from fresh plant residues; then soil biota decompose the VLF and the partially degraded plant parts move into the LF SOM. Plant parts decompose further in the LF, becoming microaggregates incorporated into the heavy fraction HF SOM. Very light fraction SOM consists of the freshest plant residue inputs to soil and decomposes relatively quickly. The LF pool, which is considered to be one of the most labile pools of SOM, consists largely of incompletely decomposed organic residues and provides a source of energy for soil microorganisms (Bending and Turner, 2009; Gosling et al., 2013). The HF is more stable, and consists of recalcitrant OC (McLauchlana and Hobbie, 2004). The quantity

of HF is closely associated with long-term C sequestration (Lorenz et al., 2007; Neff et al., 2002). Understanding the VLF, LF and HF pools provides important information about the fate of newly derived C for soil C storage.

Isotope ^{13}C and ^{14}C techniques have been successfully used to track plant C into soil C pools (Cheshire and Mundie, 1990; Warembourg and Kummerow, 1991; Liljeroth et al., 1994; Meharg, 1994; Hanson et al., 2000). Variations in ^{13}C and ^{14}C are usually expressed in terms of δ units, which are parts per thousand (‰). Generally, $\delta^{13}C$ value in the atmospheric CO_2 is -6 to -8‰, $\delta^{13}C$ values in plants range from -23‰ to -40‰, with a median value of about -27‰ (Balesdent and Mariotti, 1996). ^{14}C is a radioactive isotope and does not occur naturally in plants.

Since the 1960s, ^{14}C has been used to study C cycling in soil-plant systems (Warembourg and Kummerow, 1991; Meharg, 1994). An advantage of using ^{14}C is that no ^{14}C naturally occurs; all ^{14}C in labelled plants originates from $^{14}CO_2$. However, because ^{14}C is a radioactive material which requires extensive precautions for handling, ^{13}C has been widely used in place of ^{14}C due to its safety for handling and improvements in analytical techniques (Recous et al., 2000; Bromand et al., 2001). The two main methods used for $^{13}CO_2$ labelling plants are pulse labelling and continuous labelling.

Pulse labelling is accomplished by exposing plants to constant highly enriched $^{13}CO_2$ (usually 99 atom% $^{13}CO_2$) concertation once during crop growth (Epron et al., 2012; Leake et al., 2006). When plants fix the enriched $^{13}CO_2$ via photosynthesis, the $\delta^{13}C$ of plant parts increases. Pulse labelling is best suited to trace the dynamics of fresh assimilates of CO_2, although the amount of assimilated label during the short labelling period is generally not sufficient to achieve a detectable signal in SOM pools (Studer et al., 2014). In order to get a more integrated label, repeat-pulse labelling is used to estimate the plant C as it becomes incorporated into different soil C pools (Bromand et al., 2001). Repeat-pulse labelling involves exposing the plants to $^{13}CO_2$ more than once during the growing period. One method that produces enriched $^{13}CO_2$ is from the reaction between a solution of ^{13}C-enriched $NaH^{13}CO_3$ (33% atom ^{13}C) and HCl (Sangster, 2010). After plants were labelled for six weeks (1.5h per week), the average $\delta^{13}C$ value of stem and leaf in labelled field pea were 132.7‰ and 138.0‰ (Sangster, 2010), which were markedly higher than the $\delta^{13}C$ values of plants grown in natural abundance conditions.

Continuous labelling involves exposing plants to a constant source of labelled CO_2 (Bromand et al., 2001). The extremely high cost of the ^{13}C-enriched CO_2 makes it unsuitable for large experiments or for continuous labelling. Therefore, a continuous labelling system using ^{13}C depleted CO_2 has been proposed to track C movement in plant-soil systems (Cheng and Dijkstra, 2007). This method works by using commercially available, inexpensive CO_2 produced from natural gas which has a $\delta^{13}C$ value of -40‰ to -55‰ (Cheng et al., 2005). When plants assimilate the depleted $^{13}CO_2$ through photosynthesis, the $\delta^{13}C$ value in the labelled plants decreases.

The objective of this study was to (1) evaluate the effectiveness of a continuous labelling with depleted $^{13}CO_2$; and (2) use the depleted $^{13}CO_2$ labelling method to estimate C contribution to SOM pools of three pulse crops (chickpea, lentil and pea) in different rotation sequences.

4.4 Materials and Methods

4.4.1 Soil collection and experimental design

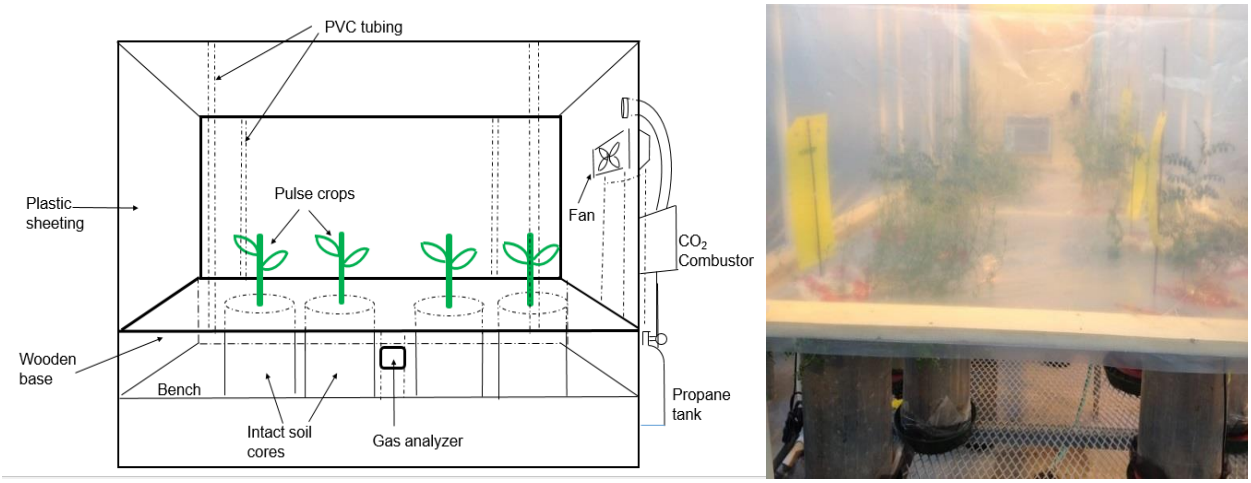
The soil collection and experimental design were the same as reported in Chapter 3 (Section 3.4.1 and 3.4.2). Briefly, soil cores (20 cm dia. by 30 cm deep) were collected from three rotations (pea-wheat, lentil-wheat and chickpea-wheat) from Agriculture and Agri-Food Canada's Semiarid Prairie Agriculture Research Centre located at Swift Current (AAFC-SPARC) (Table 4.1). Additional cores from two rotations (wheat-canola and canola-wheat) were collected from a commercial farm field located at Central Butte, SK. All rotations had completed the wheat or canola phase of the rotation the previous year. Three pulse crops (chickpea, lentil and pea) were grown in different rotation sequences (Table 4.1). Experiments 1 and 2 were established in the beginning of March and September, 2013, respectively. Seeding and inoculation was the same as described in Chapter 3 (Section 3.4.2).

Table 4. 1. Cropping sequence of intact soil cores collected from a field study at AAFC, Swift Current and commercial farm field at Central Butte. Year 1 and 2 are crops grown in the field prior to the year 3 pulse crops grown in the cores extracted in fall of year 2.

Experiment	Cropping Sequence			Field site
	Year 1	Year 2	Year 3	
1	Chickpea	Wheat	**Chickpea**	AAFC
1	Lentil	Wheat	**Lentil**	AAFC
1	Pea	Wheat	**Pea**	AAFC
1	Pea	Wheat	**Lentil**	AAFC
1	Pea	Wheat	**Chickpea**	AAFC
2	Wheat	Canola	**Chickpea**	Central Butte
2	Wheat	Canola	**Lentil**	Central Butte
2	Wheat	Canola	**Pea**	Central Butte
2	Wheat	Canola	**Wheat**	Central Butte
2	Canola	Wheat	**Chickpea**	Central Butte
2	Canola	Wheat	**Lentil**	Central Butte
2	Canola	Wheat	**Pea**	Central Butte
2	Canola	Wheat	**Wheat**	Central Butte

4.4.2 Continuous labelling with depleted $^{13}CO_2$

The plants were grown in a plastic tent and exposed to $^{13}CO_2$-depleted conditions. The tent (427cm×152 cm×168 cm) was built on a bench top in the greenhouse (Fig. 4.1). The tent frame was build out of polyvinyl chloride (PVC) tubing (JM Eagle, CA, US) mounted on a wooden base. A low-density polyethylene (LDPE) sheet with a 0.15 mm thickness (Guntap, TX, US) was attached to the PVC frame to completely enclose the plants, such that only the aerial portion of the plant was exposed to the depleted $^{13}CO_2$. The base of the plastic was secured to the wooden base by hoop & loop tape (Velcro, NH, US). The sheet allowed sunlight to penetrate for photosynthesis. The ^{13}C-depleted CO_2 was generated by combusting propane (Manchester Tank, Tillsonburg, ON, CA) using a burner (HydroGen Pro Water Cooled CO_2 Generator; Hydro Innovations, Boulder, CO). The propane tank was located outside the tent and the burner connected to the tent with a sheet-metal duct pipe (LL Building Products Inc., Burgaw, NC). Carbon dioxide was circulated throughout the tent using a household fan which was supported by a wooden base outside the tent.



(a) (b)

Figure 4.1. (a) Schematic design for $^{13}CO_2$ atmospheric labelling (b) Photograph of experiment setup for $^{13}CO_2$ atmospheric labelling.

Four weeks after seeding, soil cores were moved to the bench with the tent. A small opening in plastic allowed the plant shoots to protrude into the tent. Once the shoot was positioned into the tent, the small opening was sealed using masking tape (Uline, ON, Canada). Thus, the soil surface was isolated from the $^{13}CO_2$ atmosphere. Labelling commenced immediately after moving the soil cores. During the labelling procedure, the total $^{13}CO_2$ concentration was maintained around 1600-2800 ppm. The CO_2 concentration was monitored using an infrared gas analyzer (C.A.P. PPM-3 CO_2 Controller and Digital Monitor 120v, R&M Supply Inc., Perris, CA). For Experiment 1, treatments were replicated three times (5 crop rotation * 3 replicates). For Experiment 2, treatments were replicated four times (8 crop rotation *4 replicates). All plants were watered throughly every day before germination, then watered throughly once every two days. Soil cores were arranged randomly on the bench.

4.4.2.1 Natural abundance experiments

Natural abundance experiments were established in a different room in the greenhouse at the same time, and served as controls for the depletion experiments. Plants grown for natural abundance experiments did not receive depleted $^{13}CO_2$ labelling.

4.4.3 Sample preparation and data collection

4.4.3.1 Plant processing

Plants were harvested 15 weeks after seeding. The harvested plants were dried in a forced air oven at 50ºC, and then separated into seeds, pods, leaves, and stems. The weight of each plant part was determined. Large roots were picked out from entire cores using tweezers. All roots were washed, dried and weighed. All plant parts (seeds, pods, leaves, stems and roots) were ground using a Wiley mill then finely ground with a ball mill grinder.

4.4.3.2 Soil processing

In Experiment 1, soil cores were divided into three parts: the upper soil (0-10 cm), middle (10-20 cm) and lower (20-30cm) soil. A subsample of soil (approximately 600 g), from which large roots were removed, was collected from the upper, middle and lower soil core. For Experiment 2, a subsample of soil (approximately 600 g) was collected only from the upper 10 cm of soil after visible roots were removed. All soil samples were air dried. Subsamples of air dried soil (100 g) were finely ground with a ball mill grinder.

Additional subsamples of air dried soil (100 g) were weighed for soil fractionation. For both experiments, the LF and HF in soil samples were separated from the soil with a dense liquid (sodium iodide; NaI) following Gregorich (2007). Light fraction SOM was fractionated into VLF and LF with deionized water only in Experiment 2 (Gregorich, 2007). All extracted soil fractions (VLF, LF and HF) were oven dried at 40 ºC and finely ground by mortar and pestle.

4.4.3.3 Mass-spec analysis

All finely ground plant samples, soil sample and soil fraction samples were analyzed for %C, %N and $\delta^{13}C$ by combusting samples with a Costech Elemental Combustion system (Costech Analytical 191 Technologies, Inc.) coupled to a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.).

4.4.3.4 Calculations

The C mass for each plant part was calculated as:

C mass in plant part = %C in plant part × dry biomass of plant part (Eq. 4.1)

where plant parts were seeds, pods, stems leaves and roots. C mass in straw was the sum of C mass in pods, stems and leaves.

Change in $\delta^{13}C$ between natural abundance and ^{13}C depletion in soil fractions was calculated as:

$$\Delta\delta^{13}C = \delta^{13}C_{\text{soil fraction from plants grown in natural abundance conditions}} - \delta^{13}C_{\text{soil fraction from plants grown in depleted conditions}} \quad \text{(Eq. 4.2)}$$

4.4.4 Statistical analysis

Data were checked for normality with the Shapiro - Wilk test (P>0.05) and homogeneity of variance with Bartlett's test (P>0.05). For Experiment 1, an analysis of variance (ANOVA) was performed with the $\Delta\delta^{13}C$ values in soil fractions, %C of soil fractions, %N of soil fractions, C:N ratio of soil fractions and C mass of plant parts with different crop rotations. Means comparisons of $\Delta\delta^{13}C$ values in soil fractions, %C of soil fractions, %N of soil fractions, C:N ratio of soil fractions and C mass were running using Tukey's Honestly Significant Difference test. For Experiment 2, a 2-way ANOVA was performed to check differences in the $\Delta\delta^{13}C$ values in soil fractions, %C of soil fractions, %N of soil fractions, C:N ratio of soil fractions and C mass for the plant part with growing crops (chickpea, lentil pea and wheat) and preceding crops (wheat and canola) as the two factors. All statistical testing was performed using the statistical program R Foundation for Statistical Computing version 2.10.0 (R Development Core Team, 2010). All tests were declared significant at $P \leq 0.05$.

4.5 Results

4.5.1 $\delta^{13}C$ of plant parts and SOM fractions

Plants grown in the depleted $^{13}CO_2$ conditions had markedly lower $\delta^{13}C$ values than plants grown under natural abundance conditions (Figure 4.2 and Figure 4.3). The $\delta^{13}C$ ranges of plants under depleted $^{13}CO_2$ conditions and natural conditions, were approximately -31 to -50‰ and -20 to -30‰, respectively. In Experiment 1 (Figure 4.2), pea was the best labelled (most negative $\delta^{13}C$), in turn, chickpea obtained an intermediate label, followed by lentil with the least label (least negative $\delta^{13}C$). For Experiment 2 (Figure 4.3), each plant part had similar $\delta^{13}C$ value among pulse crops (chickpea, lentil and pea). The wheat obtained a slightly heavier labelling than the pulse crops. Generally, with the exception of lentil in Experiment 1, aboveground plant parts

(seed, pod, stem and leaf) had similar patterns of $\delta^{13}C$ in depleted $^{13}CO_2$ and natural abundance conditions. However, the roots were less labelled than aboveground plant parts, and showed different patterns from natural abundant plants. This situation also occurred in pea grown for Experiment 1.

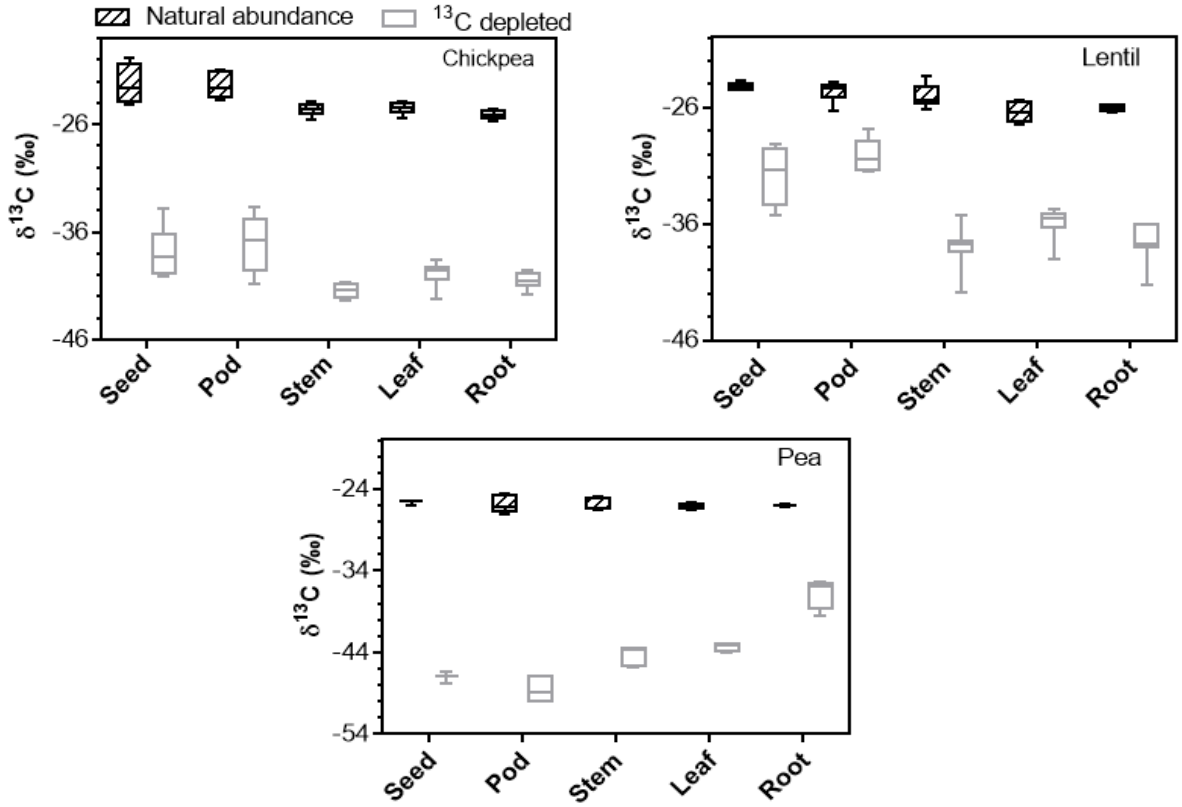


Figure 4. 2. The $\delta^{13}C$ value of chickpea (n=6), lentil (n=6) and pea (n=3) plant parts under natural abundance and depleted $^{13}CO_2$ conditions for Experiment 1. The chickpeas were in sequence with chickpea-wheat-chickpea (3 replicates) and pea-wheat-chickpea (3 replicates) rotations. The lentils were in sequence with lentil-wheat-lentil (3 replicates) and pea-wheat-lentil (3 replicates) rotations. The peas were in sequence with pea-wheat pea (3 replicates) rotation. The box is comprised of the 75th percentile, median, and 25th percentile, while the upper and lower whiskers are the maximum and minimum, respectively.

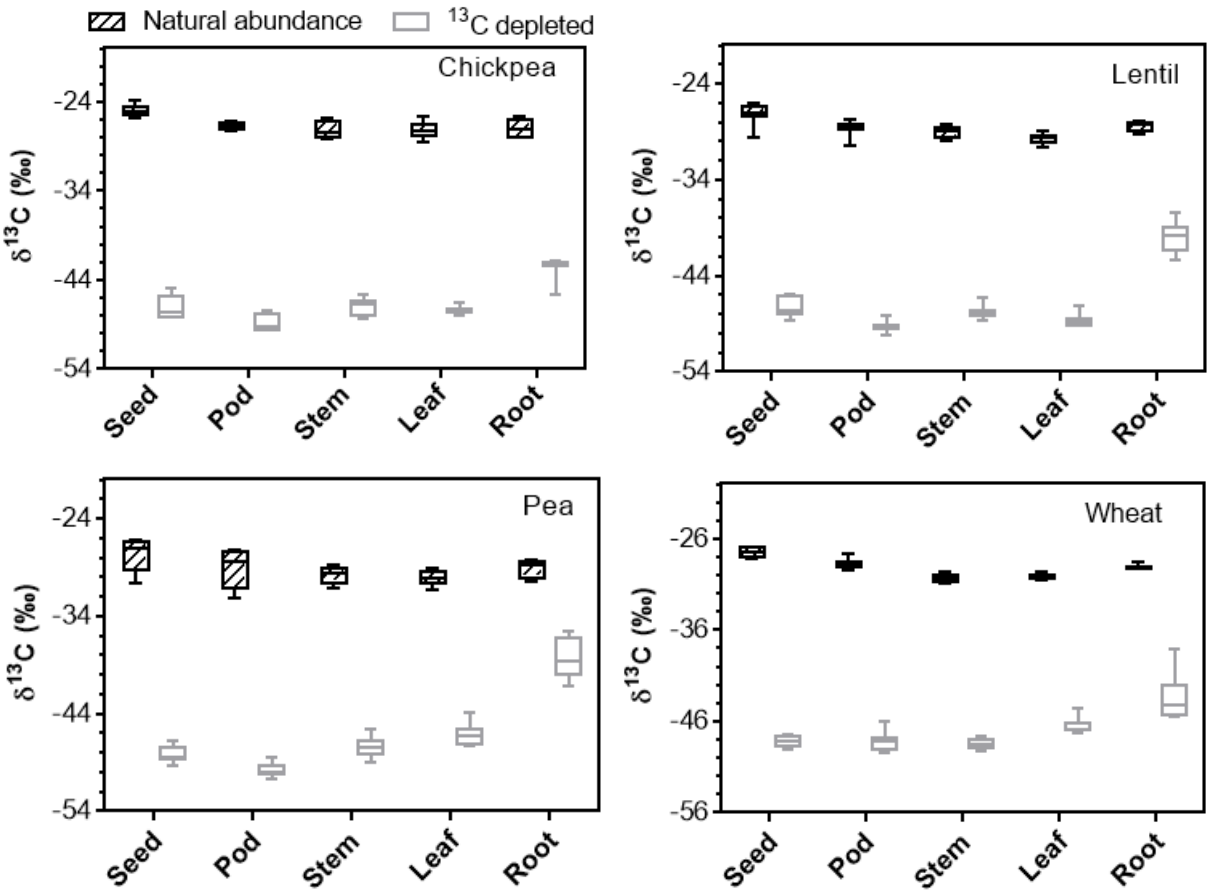


Figure 4.3. The $\delta^{13}C$ value of chickpea (n=8), lentil (n=8), pea (n=8) and wheat (n=8) plant parts under natural abundance and depleted $^{13}CO_2$ conditions for Experiment 2. The chickpeas, lentils, peas and wheats were in sequence with wheat-canola (4 replicates) and canola-wheat (4 replicates) rotations. The box is comprised of the 75th percentile, median, and 25th percentile, while the upper and lower whiskers are the maximum and minimum, respectively.

The difference between the $\delta^{13}C$ value in soil fractions from plants grown in the depleted conditions and natural abundance conditions (Appendix) was calculated. A negative value in Δ $\delta^{13}C$ suggests the movement of ^{13}C from plants to the soil (Table 4.2 and Table 4.3). In Experiment 1, the difference between $\delta^{13}C$ in LF, HF and bulk soil from plants grown in depleted $^{13}CO_2$ conditions and plants grown in natural abundance conditions did not differ among different rotation sequences (Table 4.2). The VLF from plants under depleted conditions had more negative $\delta^{13}C$ values than VLF from natural abundant plants (Table 4.3). In addition, the VLF from chickpea tended to have a larger difference in $\delta^{13}C$ value between depleted $^{13}CO_2$ and natural abundance conditions compared to the VLF from pea and lentil. For Experiment 2, the Δ δ ^{13}C in soil fractions were not affected by different preceding crops in the sequence (Table 4.3).

Table 4.2. Difference between $\delta^{13}C$ (‰) in soil fractions from plants grown in depleted $^{13}CO_2$ conditions and plants grown in a natural abundance conditions in different rotation sequences in Experiment 1. Soil fractions are the light fraction (LF), heavy fraction (HF) and bulk soil. A negative value indicates that the soil fraction is depleted in ^{13}C relative to its natural abundance counterpart; a positive value indicates enrichment in ^{13}C.

Rotation sequence	**Soil fractions**		
	LF	HF	Bulk soil
		Δ $\delta^{13}C$ (‰)	
CP-W-**CP**†	-0.05 (0.35) ‡	-0.17 (0.58)	-0.29 (0.04)
L-W-**L**	0.00 (1.29)	0.10 (0.77)	0.04 (0.31)
P-W-**P**	0.00 (0.39)	0.00 (0.92)	-0.42 (0.36)
P-W-**CP**	-0.27 (0.42)	-0.14 (0.22)	0.18 (0.65)
P-W-**L**	0.00 (0.46)	0.00 (0.27)	-0.34 (0.41)
P-value	0.983	0.981	0.494

† CP: chickpea; W: wheat; L: lentil; P: field pea.

‡ Same letter following mean (n=3) indicate no significant difference within each soil fraction following different rotations (P<0.05) according to Tukey's HSD test.

Table 4.3. Difference between $\delta^{13}C$ (‰) in soil fractions from plants grown in a depleted $^{13}CO_2$ conditions and plants grown in a natural abundance conditions following canola and wheat in Experiment 2. Soil fractions are the very light fraction (VLF), light fraction (LF), heavy fraction (HF) and bulk soil. A negative value indicates that the soil fraction is depleted in ^{13}C relative to natural abundance counterpart; a positive value indicates enrichment in ^{13}C.

Rotation sequence	**Soil fraction**			
	VLF	LF	HF	Bulk soil
	$\Delta\ \delta^{13}C$ (‰)			
W-CNL-**CP**†	-3.97 (1.78)‡	-0.13 (0.32)	-0.04 (0.75)	-0.13 (1.10)
CNL-W-**CP**	-3.70 (0.49)	-0.19 (0.15)	-0.24 (0.29)	-0.22 (0.29)
W-CNL-**L**	-0.34(0.60)	-0.73 (0.59)	0.27 (0.45)	0.32 (0.51)
CNL-W-**L**	-1.98 (0.31)	-0.41(0.42)	-0.05 (0.45)	-0.22 (0.29)
W-CNL-**P**	-0.36 (0.24)	0.18 (0.57)	-0.73 (0.10)	-0.57 (0.10)
CNL-W-**P**	-0.69 (0.27)	-0.63 (0.57)	0.32 (0.19)	0.36 (0.32)
W-CNL-**W**	-1.41 (0.75)	-0.47 (1.93)	-0.33 (0.52)	-1.58 (0.63)
CNL-W-**W**	-0.84(0.42)	-0.25 (0.76)	0.56 (0.66)	-0.31(0.24)
Summary of *P* values from ANOVA for $\Delta\ \delta^{13}C$ between grown crops and preceding crops.				
Crop	**<0.01**	0.93	0.32	**0.02**
Preceding	0.55	0.99	**0.04**	0.07
Crop × preceding	**0.03**	0.60	**0.05**	**0.01**

† W: wheat; CNL: canola; CP: chickpea; L: lentil; P: field pea.
‡ Values are means of differences ± stand errors (n=4).

4.5.2 Percentage of C and N of SOM fractions

The rotation sequences examined in Experiment 1 did not significantly affect %C, %N or C:N ratio in the LF, HF and bulk soil (Table 4.4). In contrast, canola and wheat as the preceding crop affected C and N status of the soil fractions (Table 4.5).

The %C and %N in the VLF was the highest among soil fractions (Table 4.5). In turn, the %C and %N in the LF was higher than in the HF. In terms of crops, chickpea tended to have higher %C and %N in the VLF than lentil and pea. Soil under crops following canola had a higher %C and %N in the VLF than under crops following wheat, but had a lower %C and %N in the LF than under crops following wheat.

No statistical differences were found in C:N ratio of VLF, LF and HF among crops and preceding crops (Table 4.5). A decreasing trend in C:N ratio from VLF to HF was obtained. The C:N ratios of the VLF which ranged from 12.0 to 12.5, were higher than the C:N ratios in LF (9.19 to 10.3). In turn, C:N ratios in the HF were lower than in the LF, with average values ranging from 8.76 to 9.04.

Table 4.4. Percentage of carbon (%C) and nitrogen (%N), carbon to nitrogen ratio (C:N ratio) in soil light fraction (LF), heavy fraction (HF) under chickpea (CP), lentil (L) and pea (P) grown following different rotation in the depleted $^{13}CO_2$ conditions.

Rotation sequence	**Soil fractions**								
	LF			HF			Bulk Soil		
	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N
CP-W-**CP**†	3.69 (0.96) ‡	0.32 (0.07)	11.4 (0.34)	1.17 (0.24)	0.14 (0.02)	8.64 (0.38)	1.03 (0.24)	0.12 (0.02)	8.44 (0.49)
L-W-**L**	3.52 (0.95)	0.29 (0.05)	12.1 (1.55)	1.11 (0.17)	0.13 (0.02)	8.69 (0.21)	1.02 (0.01)	0.12 (0.01)	8.57 (0.38)
P-W-**CP**	3.42 (0.18)	0.29 (0.02)	11.8 (0.29)	1.06 (0.07)	0.12 (0.01)	8.67 (0.21)	1.01 (0.08)	0.11 (0.01)	8.58 (0.11)
P-W-**L**	4.24 (0.02)	0.33 (0.01)	12.7 (0.28)	1.17 (0.17)	0.13 (0.01)	8.75 (0.34)	1.10 (0.13)	0.13 (0.01)	8.57 (0.29)
P-W-**P**	4.16 (0.76)	0.33 (0.04)	12.7 (0.85)	1.17 (0.12)	0.13 (0.01)	8.84 (0.18)	1.04 (0.03)	0.12 (0.00)	8.59 (0.16)
P-value	0.162	0.615	0.296	0.872	0.839	0.902	0.766	0.818	0.964

† CP: chickpea; W: wheat; L: lentil; P: field pea.

‡ Values are means ± standard errors (n=3). Same letter indicates no significant difference (P>0.05) using Tukey's HSD test.

Table 4.5. Percentage of carbon (%C) and nitrogen (%N), carbon to nitrogen ratio (C:N ratio) in the soil very light fraction (VLF) light fraction (LF), heavy fraction (HF) and bulk soil under chickpea (CP), lentil (L), pea (P) and wheat (W) grown following canola (CNL) and wheat (W) in depleted $^{13}CO_2$ conditions.

Rotation sequence	Soil fractions											
	VLF			**LF**			**HF**			**Bulk soil**		
	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N
W-CNL-**CP**†	16.8 (0.9)‡	1.36 (0.10)	12.3 (0.3)	3.88 (0.58)	0.40 (0.04)	9.66 (0.34)	1.77 (0.07)	0.20 (0.01)	9.04 (0.18)	1.37 (0.09)	0.15 (0.01)	9.32 (0.59)
CNL-W-**CP**	15.8 (1.4)	1.26 (0.07)	12.5 (0.4)	4.37 (0.41)	0.45 (0.03)	9.78 (0.30)	2.19 (0.27)	0.25 (0.03)	8.91 (0.05)	1.41 (0.12)	0.16 (0.01)	8.96 (0.22)
W-CNL-**L**	15.7 (2.3)	1.28 (0.16)	12.2 (0.4)	3.94 (0.46)	0.40 (0.10)	9.19 (0.50)	1.94 (0.34)	0.19 (0.02)	8.86 (0.13)	1.26 (0.11)	0.14 (0.01)	9.03 (0.26)
CNL-W-**L**	12.4 (3.3)	1.03 (0.25)	12.0 (0.4)	3.79 (0.24)	0.39 (0.02)	9.61 (0.14)	1.91 (0.38)	0.21 (0.04)	8.93 (0.24)	1.26 (0.09)	0.14 (0.01)	8.96 (0.21)
W-CNL-**P**	16.0 (2.0)	1.33 (0.15)	12.0 (0.4)	3.91 (0.42)	0.39 (0.03)	10.1 (0.7)	1.85 (0.21)	0.20 (0.03)	8.99 (0.25)	1.31 (0.17)	0.14 (0.02)	9.46 (0.91)
CNL-W-**P**	15.5 (1.7)	1.27 (0.13)	12.2 (0.3)	4.86 (0.90)	0.48 (0.07)	10.1 (0.4)	1.90 (0.27)	0.22 (0.03)	8.80 (0.09)	1.21 (0.05)	0.14 (0.01)	8.89 (0.13)
W-CNL-**W**	18.3 (1.8)	1.46 (0.14)	12.5 (0.5)	3.90 (0.49)	0.38 (0.04)	10.4 (1.2)	1.78 (0.13)	0.18 (0.01)	9.04 (0.31)	1.39 (0.10)	0.14 (0.01)	9.92 (1.51)
CNL-W-**W**	16.2 (1.3)	1.35 (0.15)	12.1 (0.8)	4.87 (0.90)	0.52 (0.11)	9.50 (0.64)	1.94 (0.22)	0.22 (0.02)	8.76 (0.20)	1.28 (0.04)	0.14 (0.00)	8.89 (0.13)

Summary of *P-value* of ANOVA analysis between grown crops and preceding crops

	VLF			LF			HF			Bulk Soil		
	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N	%C	%N	C:N
Crop	**0.023**	**0.029**	0.48	0.32	0.47	0.11	0.79	0.54	0.99	**<0.01**	0.46	0.018
Preceding	**0.020**	**0.020**	0.77	**0.015**	**<0.01**	0.74	0.12	**<0.01**	0.16	0.69	**<0.01**	0.012
Crop × Preceding	0.51	0.65	0.45	0.26	0.30	0.19	0.37	0.59	0.34	0.39	0.06	0.12

† W: wheat; CNL: canola; CP: chickpea; L: lentil; P: field pea.

‡ Values are means ± standard errors (n=4).

4.5.3 Estimated C mass

The residue C mass (straws and roots), which is an indication of the quantity of C input to soil, has the potential to be used for estimating the impact on SOC status after this growing season. Among crops, the highest C mass in seed occurred in pea (Figure 4.4 and Figure 4.5). The C mass of seed was low in chickpea and lentil (Figure 4.4 and Figure 4.5), which was probably due to differences in maturity among the three pulse crops. After harvesting, seeds were removed, the C mass distribution pattern in the straw of plants grown under $^{13}CO_2$ depleted conditions was different from that of the natural abundance pattern in plants. In general, pea produced the highest C mass in straw (Figure 4.4 and Figure 4.5b). Comparing the C mass in roots, chickpea had higher root C mass than lentil and pea (Figure 4.4a and Figure 4.5a). Therefore, the chickpea may contribute more C to soil through rhizodeposition compared to lentil and pea during growth.

In terms of rotation sequence effects, lentil grown in the lentil-wheat-lentil sequence produced higher C mass in seeds and residues (straw and root) than lentil grown in the rotation with pea (pea-wheat-lentil) (Figure 4.4). There was a tendency that pulse crops following wheat had higher C mass in seeds and residues (straw and root) than those following canola (Figure 4.5).

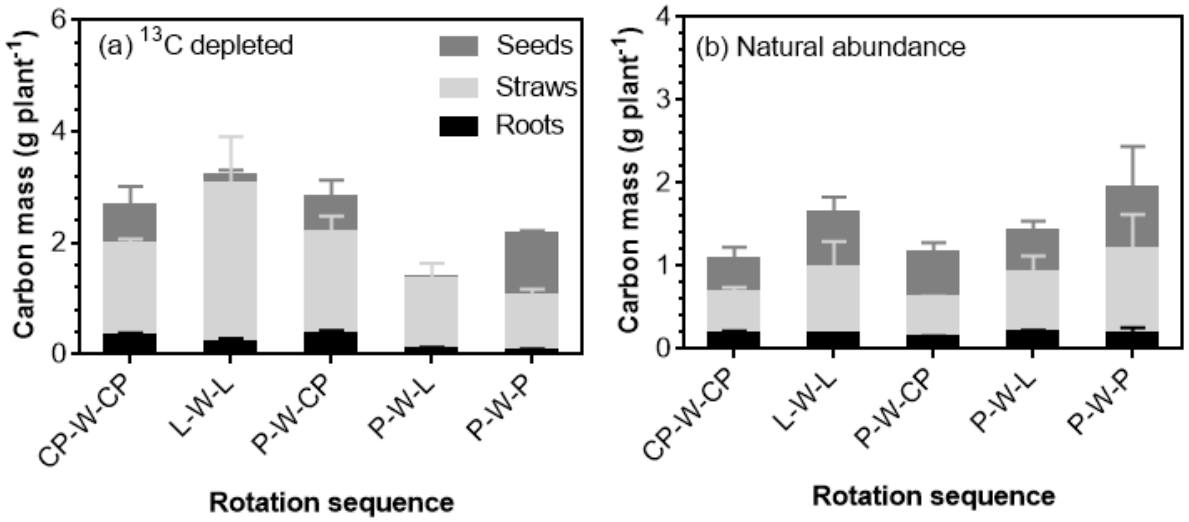


Figure 4. 4. Carbon mass in the plant parts for chickpea (CP), lentil (L) and pea (P) grown in different rotations in the (a) depleted $^{13}CO_2$ and (b) natural abundance conditions. W: wheat. Straws inclded pods, stems and leaves. Bars represent means ± standard errors (n=3).

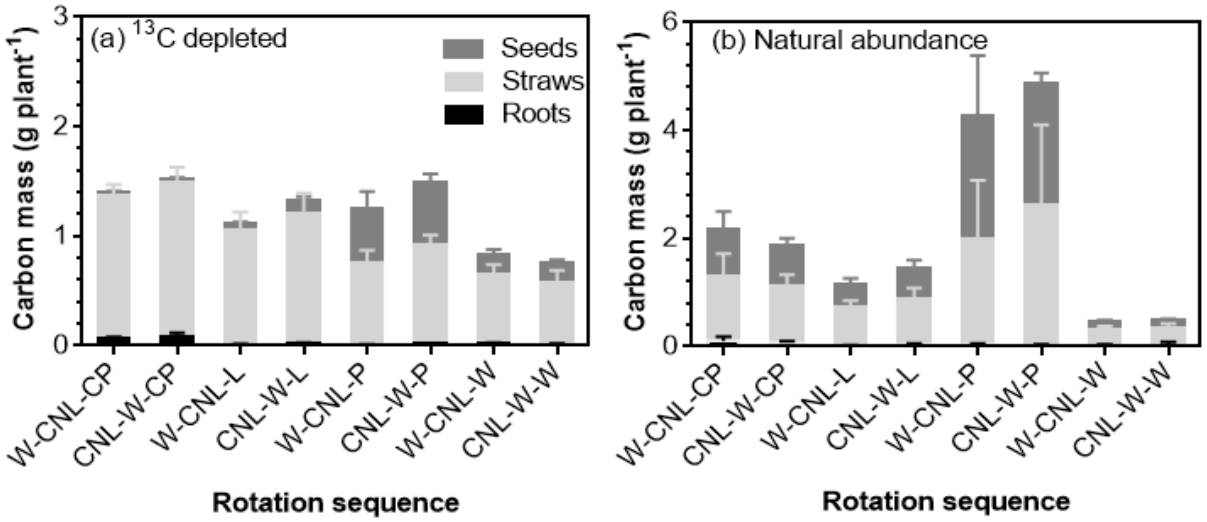


Figure 4. 5. Carbon mass in plant parts of chickpea (CP), lentil (L), pea (P) and wheat (W) following canola (CNL) and wheat (W) in the (a) depleted $^{13}CO_2$ and (b)natural abundance conditions. Straws inclded pods, stems and leaves. Bars represent means ± standard errors (n=4).

4.6 Discussion

The continuous labelling with depleted $^{13}CO_2$ was effective in depleting ^{13}C in all the plants (Figure 4.2 and Figure 4.3). However, compared to the theoretical value for ^{13}C depletion, plants were not sufficiently depleted. Propane is naturally depleted in ^{13}C, having a $\delta^{13}C$ of approximately -46‰ (Cheng and Dijkstra, 2007). During photosynthesis, C_3 plants discriminate against ^{13}C by approximately 19‰ (atmosphere CO_2 -8‰; in plant -27‰) thus a C_3 plant photosynthesizing atmosphere CO_2 (-8‰) has a $\delta^{13}C$ value of approximately -27‰. Plants photosynthesizing CO_2 generated from propane should have a theoretical $\delta^{13}C$ of -65‰ (-46 + -19‰), indicating that the majority of the C fixed in plants is from propane. In this study, similar to the $\delta^{13}C$ value of plants grown in natural conditions, the average $\delta^{13}C$ value of natural abundance plants varied from -23 to -30 ‰. The range of $\delta^{13}C$ value of plants grown in the depleted $^{13}CO_2$ was approximately -30 to -50‰, indicating a ^{13}C-depletion within plant parts. The labelling was not conducted throughout the full growth cycle, and started 4 weeks after seeding.

The homogenous labelling of C pools provides an integrated result of C cycling (Studer et al., 2014). One prerequisite for estimating residue-C movement through C pools is uniform label distributing through at the plant (Sangster, 2009). The decomposition rate and extent may vary among chemical compounds that made up crop residues (Trinsoutrot et al., 2000). Therefore, the contribution of ^{13}C to the soil C pools can differ, which would result in erroneous estimates of residue-C contribution to the soil. In this current study, the roots of all crops were the least depleted (i.e. had least negative $\delta^{13}C$ values) of the plant parts (Figure 4.3). This may result in an underestimation of the contribution of belowground residue C to soil C pools. The weakest labelling among plant parts is likely due to rapid root growth in the initial phase of crop development; i.e. labelling begun at 4 weeks after seeding. The distribution of ^{13}C within the plant would be enhanced if labelling was started as early as possible (Thompson 1996; Bromand et al., 2001). Starting the labelling procedure earlier would be challenging because young plants have to be sealed into the tent. Even though non-homogeneity of ^{13}C-depletion in the various plant parts was obtained following the continuous labelling with depleted $^{13}CO_2$, a similar distribution pattern of ^{13}C in the aboveground shoots (seed, pod, stem and leaf) was found compared to the natural abundance value in plants (Figure 4.2 and 4.3).

The variability in ^{13}C-depletion among plant parts observed in the experiments may be attributed to unavoidable plant variation. Because intact soil cores were used to grow the plants, the soil between core samples was inherently heterogeneous. Although plants were grown in a single core, they were not uniform between cores and within cores with some having a greater biomass than other plants even in the same core. In addition, location of the cores may have contributed to the variation of ^{13}C-depletion. That is, some cores were within a higher concentration of $^{13}CO_2$ because they were closer to the side of the tent where the CO_2 generator released $^{13}CO_2$. This effect was minimized by circulating the generated $^{13}CO_2$ with a fan, but insufficient or variable circulation may still have resulted in a heterogeneous atmosphere. Plants in the tent might receive different amounts of light during labelling affecting the rate of photosynthesis and thereby metabolism of ^{13}C by the plants. Furthermore, crop species with different genotypes might impact ^{13}C-depletion (Dawson et al., 2002). In both experiments, lentil under depleted $^{13}CO_2$ conditions had overall lower ^{13}C-depletion than chickpea and pea. This might be attributed to slower growth of lentil with less biomass. Photosynthesis in lentil was not as rapid as other crops, resulting in the lower incorporation of the depleted $^{13}CO_2$ than in the chickpea and pea.

This continuous labelling with depleted $^{13}CO_2$ resulted in a similar pattern of relative ^{13}C distribution within plants as the repeat-enriched $^{13}CO_2$ pulse labelling conducted by Comeau (2012). In Comeau's study, plants were exposed to enriched $^{13}CO_2$ conditions (33% atom$^{13}CO_2$) labelled 2 hours a week for eight weeks. A similar small variation existed among aboveground plant parts. The roots also obtained less label than aboveground plants parts. Compared to repeat ^{13}C-enriched CO_2 labelling, the design of the continuous labelling with depleted $^{13}CO_2$ was simple and the expense was lower than other enriched $^{13}CO_2$ labelling methods. In addition, using an automated combustion system controlled by a gas analyser resulted in this method was efficient and not labour-intensive than the repeat pulse labelling method.

According to the dynamic model proposed for decomposition of residues and their incorporation into different soil fractions (Comeau et al., 2013), the fresh residue C is first incorporated into the VLF. The VLF is further decomposed and partially enters into the LF. Additional decomposition occurs in the LF, with the residue then fixed into the HF. In my study, the movement of ^{13}C from plants to soil through rhizodeposition was obtained. However, the

amount of ^{13}C transfer from rhizodepostion to SOM fraction pools was too small to calculate due to small $\Delta\ \delta^{13}C$ value (Table 4.2 and 4.3). Nevertheless, it was found that most of the newly derived C was in the VLF (most negative $\Delta\ \delta^{13}C$ value among SOM fractions). In addition, a higher %C was obtained in the VLF than in the LF and HF (Table 4.5). The higher %C in the VLF suggested that most rhizodeposit-C was in the VLF. In the VLF, the higher %C indicated more energy resources for microorganisms, thereby stimulating an increase in microbial metabolites for residue decomposition (Glenn et al., 2011). In this current study, the C:N ratio in the VLF, LF and HF ranged from 12.0 to 12.5, 9.2 to 10.4, and 8.8 to 9.0, respectively. Compared to the C:N ratio in LF and HF, the higher C:N ratio in VLF suggested that the VLF was composed of plant residues in the early stages of decomposition (Liao et al., 2006; Freixo et al., 2002). Johnson et al. (2005) states that high C:N ratios occur in soil during organic C degradation. During decomposition, a higher consumption of C than N occurs, resulting in a relative increase in N concentration. Therefore, an increasingly narrow C:N ratio was obtained from VLF to LF to HF (Gregorich and Beare, 2007; Comeau, 2013).

Among the VLF fractions, the soil used for growing chickpea had more ^{13}C depleted and tended to have a higher %C and %N than soil used for lentil and pea (Table 4.5). Due to the higher root C biomass produced by chickpea (relative to lentil and pea), the rhizodeposited-C amount from chickpea should be greater.

The newly rhizodeposited-C did not significantly affect the LF and HF pools. In my study, the $\Delta\ \delta^{13}C$ value between LF and HF from plants in depleted $^{13}CO_2$ conditions and natural abundance conditions was very small (Table 4.2 and Table 4.3). In addition, crops did not affect %C and %N in the LF and HF (Table 4.4 and Table 4.5). This is likely due to the amount of the rhizodeposits may be not large enough to influence the $\delta^{13}C$ value in the LF and HF. In addition, the rhizodeposits had not enough time for VLF to undergo humification.

When canola was grown as the preceding crop, the soil in which the succeeding pulse crop was grown maintained a higher %C and %N in the VLF than when preceded by wheat. In contrast, the LF in soil growing pulse crops following canola had lower %C and %N than pulse crops following wheat. The rhizodeposition from pulse crops in this growing season was likely not enough to impact soil C pools. In this current study, the canola and wheat root residues from the prior growing season made up a considerable amounts of total residue input to the soil. In the

VLF, the significant difference in %C and %N with different preceding crops may be associated with crop species. The decomposition of the roots is more closely related to the morphology of the roots. Wheat is a monocot and has a fibrous root system and roots have scattered vascular bundles. Canola is a eudicot plant with a taproot and vascular bundles in a ring. Compared to the adventitious roots of a monocot (wheat), the taproot of a dicot crop (canola) is known to be stronger (Evert et al., 2006). Therefore, the roots of wheat may decay faster than the root of canola. Consequently, a decomposition of residue-C fixed into the LF may be faster after wheat than in soil after canola.

Although no significant difference was found in the amounts of residue-C returned to the soil (roots and straws) from the pulse crops when following different preceding crops, there was still a tendency for the amount of residue-C from pulse crops grown immediately after wheat to be slightly higher than following canola (Figure 4.4 and Figure 4.5). The impact on the growth of pulse crops following canola may be attributed to phytotoxic compounds from the canola residue and reduced populations of beneficial organisms in soil growing canola (Wanniarachchi and Voroney, 1997). I found that the amount of residue-C from wheat following canola was greater than that from in the continuous wheat (Figure 4.5). Indeed, previous studies suggested that increased diversity of crops in a rotation increases productivity (Matus et al., 1997), therefore was likely to increase residue C inputs to the soil. In contrast to the previous study, the second lentil crop twice in a lentil-wheat-lentil rotation produced a larger amount of residue-C than lentil grown in a pea-wheat-lentil rotation (Figure 4.4). This is likely due to the residual bacteria survived in soil was more efficient for nodulation in the same pulse crop, then impacting production of plants.

4.7 Conclusion

The effectiveness of the continuous ^{13}C depletion method was verified by depletion labelling plants with a significantly lower level of $\delta^{13}C$ compared to the natural abundance plants. Roots were the least depleted compared to other plant parts. However, when using this method to track the movement of C from plant to SOM pools, ^{13}C-depletion was obtained in SOM pools, but the magnitude was very small. The cropping sequence effect on soil C pools could not be examined satisfactorily using the continuous labelling with depleted $^{13}CO_2$. The newly derived belowground residue C was found in the VLF. The amount of newly derived

belowground residue C was not enough to significantly affect the LF and HF pools. Chickpea tended to contribute a greater amount of C to the VLF than pea and lentil. Further research should be conducted by applying residue of labelled plant to follow C in order to examine soil C pools (VLF, LF and HF) following a growing season.

5. GENERAL DISCUSSION AND CONCLUSION

5.1 Summary of findings

The general goal of this research was to evaluate the cropping sequence effects on BNF and C input to soils by chickpea, lentil and field pea. Chickpea, lentil and field pea were grown in various sequences with pulse crops (pulse-wheat) and without pulse crops (wheat-canola and canola-wheat) with the test pulse grown as the third crop in these sequences. This study enabled the comparison of cropping sequence on BNF and C inputs to soil by three pulse crops (chickpea, lentil and field pea) simultaneously. Dual labelling using ^{15}N and ^{13}C was conducted during the growing phase of chickpea, lentil and field pea. The ^{15}N dilution method was used to estimate BNF. A continuous labelling with depleted $^{13}CO_2$ was used in the 3rd year of the sequence to trace the ^{13}C transferred from plants to SOM pools (VLF, LF and HF).

The ^{15}N dilution method was an effective method at determining BNF by aboveground plant parts (seeds, pods, stems and leaves). However, the BNF by roots was not measurable, though the reasons for this were unknown. After exposing plants to a continuous source of depleted $^{13}CO_2$, all the plant parts were depleted in ^{13}C. By using this continuous labelling with depleted $^{13}CO_2$, we observed the movement of ^{13}C from plants to soil C pools, but were not able to quantify the amount of rhizodeposited-C released by roots. Most of the rhizodeposited-C was found in the VLF (lowest $\delta^{13}C$ and highest %C among SOM fractions). Nevertheless, residue C mass (pods, stems, leaves and roots), which represents the quantity of C input to soil, could potentially be used for estimating the impact on SOC status (Campbell et al., 2000) after a growing season. An increase in residue C input can lead to an increase in SOC level (Victoria et al., 2012).

The BNF and residue-C produced in the current study is likely to be underestimated compared to field studies (Herridge et al., 2008; Gan et al., 2009; Gan et al., 2010). Typical target seeding densities in the field for chickpea is 40 plants m^{-2}, for lentil is 120 plants m^{-2} and for pea is 80 plants m^{-2}. The seeding density in this study equalled 125 plants m^{-2}. Compared to

crops grown in fields, the relatively higher density of pulse crops (except lentil) in the current study may have resulted in higher competition for resources required for growth. Therefore, the biomass produced, the composition of which is strongly related to residue-C and BNF, may have been low in my study. Gan et al. (2009) reported residue-C from chickpea, lentil and pea was 1196, 1268, 1064 kg ha^{-1} respectively. The extrapolation of residue-C production by chickpea, lentil and pea under depleted conditions in Experiment 1 was 846, 2468, 857 kg ha^{-1}, respectively. After extrapolation, the residue-C production in Experiment 2 by chickpea, lentil and pea grown in the depleted $^{13}CO_2$ atmosphere was 578, 1268 and 659 kg ha^{-1}. Similar to residue-C production, the BNF by pulse crops was lower than global averages. The global averages for BNF by chickpea, lentil and pea are 58, 51, 86 kg N ha^{-1}, respectively (Herridge et al., 2008; Gan et al., 2010). For this current study, the extrapolated amounts of BNF in shoot (seeds, pods, stems and straws) by chickpea, lentil and pea in Experiment 1 were approximately 16, 21 and 43 kg N ha^{-1}, whereas in Experiment 2 they were approximately 20, 39, 43 kg N ha^{-1}, respectively.

Different pulse crops performed differently in rotation. With the same prior rotation sequence, even though field pea produced less biomass than chickpea, pea still fixed the greatest amount of N_2. Similar results were found in field studies conducted by Gan et al. (2010) and Herridge et al. (2008). In addition, after removing seed when harvested, pea grown under natural abundance conditions tended to have more C mass (pods, stems, leaves and roots) compared to chickpea and lentil.

This study provided interesting data on the effect of frequency of cropping chickpea and lentil in a three year rotation on BNF and residue-C mass. When cropping lentil twice alternatively with wheat (lentil-wheat-lentil), the second lentil crop fixed larger amounts of N_2 and C than when lentil was grown in sequence with pea-wheat. This was attributed to higher biomass production by lentil grown in the lentil-wheat-lentil rotations than by lentil grown in the pea-wheat-lentil rotations. Similarly, including chickpea twice in a three year rotation (chickpea-wheat-chickpea), produced a greater amount of biomass and C mass than cropping chickpea once in sequence with pea-wheat. This may be due to residual bacteria in soil forming more efficient symbioses with the same pulse crop. However, this finding is in contrast to previous studies (Matus et al., 1996; Hardarson, 2003), suggesting that increased diversity of crops in a rotation

increases productivity. It is thought that rotations that include diverse crops increase the adaptability of crops to change in environmental factors (diseases, insects and weeds). In my greenhouse study, however, these factors were controlled well.

Growing a pulse crop after canola has an adverse effect on the pulse crops. Cropping pulse crops following canola decreased the amount of BNF and residue-C input to soil compared to grow the pulses following wheat. Canola residue releases phytotoxic compounds that reduce the population beneficial soil organism population. Although a positive effect of a preceding canola crop on the subsequent crop yield has been reported (Brandt and Zentner, 1995; Soon and Clayton, 2002), at least in this Brown soil zone, pulse crops grown immediately after canola is not recommended.

5.2 Future work

This study was conducted in a greenhouse, plants were well maintained under near optimal conditions. However, in the field, plants may experience water deficient stress plus interference of weeds and infection of diseases. In addition, the soil conditions in the field may differ from soil in the small cores in the greenhouse. Therefore, to maximize the benefits of growing pulse crops in a rotation, field scale studies are needed to riddle the cropping sequence effect on BNF and C inputs to soil.

The ^{13}C depletion in this study was not sufficient for quantifying ^{13}C transformation from plants to soil C pools via rhizodeposits within a growing season. A longer labelling period and the earlier start of labelling may improve quantification of tracing the movement of ^{13}C into soil C pools. Furthermore, in order to gain a greater knowledge and understanding of SOM dynamics in the soil as influenced by cropping sequence with different pulse crops, long-term studies with labelled plant residues would be helpful.

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APPENDIX

δ ^{13}C(‰) of soil fractions of growing crops in the deleted in the depleted $^{13}CO_2$ and natural abundance atmosphere.

Appendix 1. δ ^{13}C(‰) of the soil light fraction(LF), heavy fraction (HF) and bulk soil of chickpea, lentil, field pea in the depleted $^{13}CO_2$ and natural abundance atmospheres(NA).

Rotation sequence	**LF**		**HF**		**Bulk soil**	
	Depleted	NA	Depleted	NA	Depleted	NA
CP-W-CP†	-25.7 (1.2) ‡	-25.7 (0.3)	-23.8 (0.3)	-23.9 (0.6)	-23.6 (0.4)	-23.5 (0.4)
L-W-L	-26.4 (0.6)	-25.2 (1.3)	-23.2 (1.1)	-23.6 (0.7)	-23.7 (0.3)	-23.5 (0.5)
P-W-P	-26.4 (0.3)	-25.8 (0.4)	-23.4 (0.3)	-23.1 (0.9)	-23.4 (0.5)	-23.4 (0.5)
P-W-CP	-26.2 (0.3)	-25.6 (0.4)	-23.8 (0.4)	-23.7 (0.3)	-23.3 (0.6)	-23.5 (0.1)
P-W-L	-26.0 (0.1)	-25.8 (0.5)	-23.7 (0.4)	-23.8 (0.3)	-23.7 (0.4)	-23.4 (0.2)
p-value	0.98		0.69		0.86	

† CP: chickpea; W: wheat; L: lentil; P: field pea.
‡Value are means ± standard errors (n=3)

Appendix 2. δ ^{13}C (‰)of soil the very light fraction (VLF) light fraction(LF), heavy fraction (HF) and bulk soil under chickpea, lentil, field pea and wheat grown following canola (CNL) and wheat (W) in the depleted $^{13}CO_2$ and the natural abundance atmospheres (NA).

Rotation sequence	**VLF**		**LF**		**HF**		**Bulk soil**	
	Depleted	NA	Depleted	NA	Depleted	NA	Depleted	NA
W-CNL-CP†	-31.0 (1.8) ‡	-26.6 (0.5)	-24.9 (0.5)	-24.5 (0.5)	-22.4 (0.7)	-22.3 (0.3)	-22.2 (1.1)	-22.3 (0.3)
CNL-W-CP	-29.8 (0.5)	-26.1 (1.1)	-25.2 (0.1)	-24.9 (0.9)	-24.1 (0.3)	-23.9 (0.2)	-23.9 (0.1)	-23.6 (0.4)
W-CNL-L	-26.7 (0.6)	-26.4 (0.4)	-24.1 (0.6)	-23.3 (1.3)	-22.6 (0.5)	-22.4 (0.9)	-22.2 (0.5)	-22.1 (0.7)
CNL-W-L	-27.6 (0.3)	-26.6 (1.6)	-24.2 (0.4)	-23.8 (0.4)	-23.4 (0.4)	-23.4 (0.4)	-23.2 (0.3)	-23.0 (0.6)
W-CNL-P	-26.0 (0.2)	-25.6 (1.7)	-24.2 (1.0)	-24.5 (2.1)	-22.6 (0.1)	-21.9 (0.1)	-22.1 (0.1)	-21.5 (0.4)
CNL-W-P	-26.1 (1.2)	-25.8 (1.9)	-24.8 (0.6)	-24.1 (0.6)	-23.5 (0.2)	-23.8 (0.5)	-23.2 (0.3)	-23.5 (0.4)
W-CNL-W	-27.9 (0.8)	-26.6 (0.4)	-23.0 (1.9)	-22.5 (1.6)	-22.6 (0.5)	-21.9 (0.6)	-22.4 (0.6)	-21.3 (0.7)
CNL-W-W	-27.4 (0.4)	-26.2 (0.2)	-24.3 (0.8)	-24.0 (0.5)	-22.9 (0.7)	-23.5 (0.3)	-23.5 (0.2)	-23.2 (0.4)
p-value	**<0.01**		0.45		0.30		0.39	

† W: wheat; CNL: canola; CP: chickpea; L: lentil; P: field pea.
‡Value are means ± standard errors (n=4)